CIBA FOUNDATION COLLOQUIA ON AGEING

Vol. 5. The Lifespan of Animals

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CIBA FOUNDATION

COLLOQUIA ON AGEING

VOLUME 5

The Lifespan of Animals

Editors for the Ciba Foundation

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and Cumulative Index to Volumes 1-5



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Ciba Foundation attempts in every other way possible to aid scientists, whether they be Nobel Laureates or young graduates making their first original contribution to research.

The purpose of the Ciba Foundation, which is to promote

Aesculapius) holding a medical caduceus, and three regular hexagons for chemistry. Its domicile in London is indicated by the red sword of St. Paul and the British lion; the wyvern and the crozier, symbols associated with Basle, refer to the sponsoring firm located in this ancient Swiss town.

PREFACE

EARLY in 1954 the Trustees of the Ciba Foundation designated funds over a period of five years for the encouragement of basic research relevant to the problems of Ageing One feature of this special programme was to be the organization of small international conferences, on lines familiarly known at the Foundation in regard to other subjects in medical research, to assemble and consider such information as could be gathered in [illegible]

[illegible] The Trustees now suspend the Foundation's special stimulation of work in this field The Director believes that considerable attention has been drawn to the many large gaps in knowledge which must be filled before the postponement and amelioration of senescence can be realized Research workers should now be left to their task, the size and importance [illegible]

programme of the Foundation

The Director and his co Editor wish to place on record with these proceedings the indebtedness of the Foundation and themselves to Professor Danielli and Dr. Comfort for giving life [illegible]

Ballantyne & Co Ltd for minimizing, without skimping or harassment, the delay in publication in this difficult year.

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List of those participating in or attending the Colloquium on "The Lifespan of Animals", 14th-16th April, 1959

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J Rotblat	Dept. of Physics, St. Bartholomew's Hospital Medical College, London

of the division of causes of death into two kinds, those due to chance and those due to deterioration

It was soon evident that such a relatively simple law would not represent mortality experience throughout life and subsequent developments led to the proposal of more complex mathematical relationships between age and the force of mortality and even of different relationships over different parts of the age range Thiele (1871) for example proposed

$$\mu_x = a_1 e^{-b_1 x} + a_2 e^{-b_2^2 (x-c)^2} + a_3 e^{b_3 x}$$

in which the last term is a Gompertz curve to represent old-age mortality, the first a decreasing Gompertz curve to represent the mortality in childhood, and the middle term a normal curve Perks (1932) introduced a new family of curves in the general form

$$\mu_x = \frac{A + Bc^x}{Kc^{-x} + 1 + Dc^x}$$

and rationalized this procedure with some interesting speculations on the theory of mortality He found an analogy between the "inability to withstand destruction" of Gompertz and the then current physical concept of entropy change—the measure of the time progression of a statistical group from organization to disorganization Perks also referred to the previous work of Karl Pearson who fitted overlapping curves not to the force of mortality but to the curve of deaths, the curves being intended to represent the mortality of old age middle life, youth, childhood, and infancy, the causes of death being different in these different periods of life Perks pointed out that this search for homogeneity in the pattern of causes of death might lead to endless subdivision

[illegible]

[illegible] and $d_x = \int_x \mu_x l_x \, dx$

The continuous curve of $(\mu_x l_x)$ is called the curve of deaths.

ACTUARIAL ASPECTS OF HUMAN LIFESPANS

B Benjamin

General Register Office, London

Actuaries have always been profoundly interested in the variation in the incidence of mortality with age since many of their calculations of contingencies depend upon this variation The study of this age variation in mortality has given rise to a number of hypothetical "laws" of mortality based on theories about the exertion on the human body of deleterious influences or about the wearing out of components of the body and the exhaustion of living resources These theories date from Gompertz (1825) who argued on physiological grounds that the intensity of mortality (in his terms the average exhaustion of man's power to avoid death) gained equal pro portions in equal intervals of age and Makeham (1867) who introduced a constant component as well as a logarithmically increasing component of the force* of mortality as a reflection

* It

$$\left(-\frac{l_{x+1}}{l_x}\right)$$

(4) q_x the chance of dying between exact age x and exact $x + 1$

$$\left(=\frac{d_x}{l_x}\right)$$

Note $p_x + q_x = 1$

(5)

Later, in a discussion of another paper on fitting a mathematical law to l_x of the mortality table (Ogborn, 1953), Perks suggested that reference to the probability models of the biological field and the data of simple populations was the only way in which an advance would be made in the development of a satisfactory theory of a life table It is a pity that this suggestion has not yet been exploited as, if linked with the idea of loss of biological organization, it seems to the present author to open up an important line of approach But we are straying from the objective of this historical introduction

In 1954 Phillips returned to consideration of the curve of deaths and hypothesized the existence of a basic curve of deaths 'to which all curves of deaths are, as it were, striving to attain'

Clarke s division of deaths into "anticipated" and "senescent" has been further developed by Barnett (1955 and 1958) but applied to the force of mortality, not the curve of deaths On the basis of cause of death grouping and the actual shape of the curve of observed age rates of mortality Barnett distinguished several different groups of anticipated deaths

We may now return to Clarke's paper

The earlier approach

The [illegible] ng of the [illegible] His hy[illegible] at every individual carried with him from birth a genetically endowed term of life beyond which it was impossible for him to survive, and that if we knew these terms for every member of the population we could form a frequency distribution similar to that of any other biometric quantity This distribution would represent a limiting form of the curve of deaths Clarke went further and supposed that this distribution would not shift as a whole toward later

of the curve of deaths into component curves and that it was questionable whether such subdivision could be theoretically validated Nevertheless he agreed that one important case of sudden change of composition was the rapid transition between the period of physical growth and the adult period "In the adult period we appear to be subject to a continually increasing disorganisation or 'inability to withstand destruction' while in childhood we appear on balance to gain organisation or 'ability to withstand destruction' "

While further work continued on the fitting of mathematical functions to the force of mortality, μ_x, attention was focused on the curve of deaths (i e of $\mu_x l_x$) as an alternative operand by a paper by Phillips (1935), though this was not yet to be taken up Meanwhile, taking a closer look at the concept of deterioration, Rich (1940) evolved a theory of mortality based on an analogy between degrees of health and degrees of temperature This gave rise to a health frequency distribution" (a concept close to the present view of biological normality as a dispersion of characteristics within broadly separated limits), and changes of rating within this distribution could be regarded as forces of deterioration or of recuperation A "natural law of mortality" emerged from which Makeham's and one of Perks's functions could be derived as special cases

Clarke (1950) took up again the analysis of the curve of deaths He argued that mortality improvements had not extended the natural lifespan but had only allowed more to achieve it He distinguished between "anticipated" and "senescent" deaths, the ages at death in the latter group were measures of natural lifespans and had a frequency distribution like other animal characteristics His paper formed the basis of the present work and his hypothesis will be referred to again presently

Beard (1950) also paid attention to the curve of deaths, using the incomplete gamma function as the basic analytical function

approximation to the curve of deaths (d_x is of course discontinuous while the "curve of deaths" is continuous It has been assumed that $d_x \approx \mu_{x+\frac{1}{2}} l_{x+\frac{1}{2}}$) The curve has then been treated

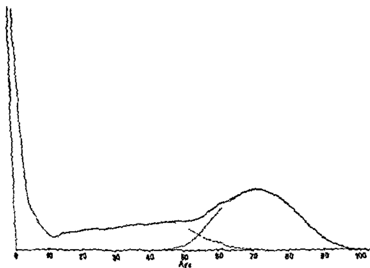


FIG 1 Curve of deaths, English Life Table No. 1 1841 Males
total deaths
senescent deaths
anticipated deaths

side of this distribution has been drawn in (broken line in the figures) to exactly mirror the right hand side It is thus assumed (unlike *Clarke*) that the biometric distribution of lifespans is symm

st

d

b ... to zero at the peak of the senescent deaths In effect it is assumed in *Fig* 3 that no deaths before

ages, i e that the modal span of life was invariant, he rejected the possibility as "intangible speculation" outside the practical framework of his study, namely the relatively short term trend of mortality Deaths were then divided into two categories, namely those which happened because the term of the lifespan had run out—senescent deaths—, and all others, whether from accident or disease, which were in fact a cutting short of the lifespan—anticipated deaths He first obtained, therefore, a limiting curve of deaths by constructing a table of mortality in which the q_x (probability of death between age x and age $x + 1$) column would consist of values (q_x^s) appropriate only to causes of death which could be regarded as senescent The next stage was to predict the pace at which actual rates of mortality would in a given future period approach those of the limiting table It is not necessary to discuss this aspect here

Clarke originally intended to define 'senescent" deaths by choosing certain degenerative diseases (e g cerebral vascular lesions, myocardial diseases, angina pectoris, arteriosclerosis, other diseases of the circulatory system, bronchitis, nephritis), but he naturally found it difficult to select disease groups with sufficiently specific reference to degeneration Ultimately therefore he arbitrarily assumed ratios of q_x^s/q_x rising from 0 05 at age 20 to 0 10 at age 40 0 20 at age 50, 0 70 at age 70, 1 00 at ages 80 and above

His limiting curve of deaths was not symmetrical There was a sharp peak at age 80 with a tailing off rapidly on one side to age 100 or so and on the other side a rapid decline to about age 60 and then a much slower tailing off to age 20

The present approach

The present author's approach has been even more arbitrary and pragmatic In a particular life table the values of d_x (deaths between age x and $x + 1$) have been plotted for every value of x in the table (Figs 1–3), thus producing an

arguable and it is proposed to adopt the latter hypothesis here.

The data used

The analysis already described was applied to three national life-tables (for both males and females, though Figs. 1–3 relate to males only):

FIG 3 Curve of deaths English Life-Table No. 11 1930–1932. Males.
——————— total deaths
senescent deaths
— — — — — — anticipated deaths

[illegible] ompiled by Dr. [illegible] It differs from [illegible] both males and females start with a radix of 100 000 births [illegible]

age 55 and all deaths after age 76 are senescent It is hoped that this brash sweeping aside of the honest testimonies of millions of medical practitioners which are recorded in the vaults of Somerset House will not give offence It is of course a moot point as to whether any death after age 76, even

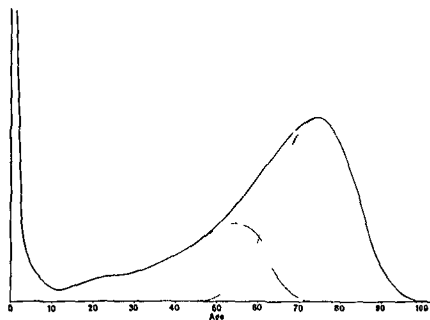


Fig. 2 Curve of deaths English Life Table No 8 1910–1912 Males

——————— total deaths

senescent deaths

– – – – – – – anticipated deaths

allegedly due to some accident, is other than of senile origin, but some deaths before age 55 are of degenerative origin and at age 55 Clarke assumed that 20 per cent of them were "senescent". Whether this degeneration is senescent in the sense of the completion of a genetically endowed lifespan or whether it is the cutting short of the span by departure from optimum environmental conditions and behaviour is at least

arguable and it is proposed to adopt the latter hypothesis here.

The data used

The analysis already described was applied to three national life-tables (for both males and females, though Figs. 1–3 relate to males only):

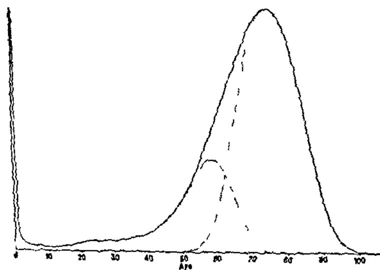


Fig 3 Curve of deaths. *English Life-Table No. 11.* 1950–1952. Males.

———— total deaths
. senescent deaths
– – – anticipated deaths

Engl ish I e T

ompiled by Dr.
It differs from
... both males and
females start with a *radix of 100,000 births*, in that Farr to facilitate the product of a "persons" table chose radices of 51,274 male births and 48,726 female births (the proportions

of males and females in the births of that period) All the ordinates of Fig 1 should therefore be almost doubled to render it comparable with Figs 2 and 3, though the general shape is correct

English Life Table No 8 The first short period table prepared by an actuary, George King, for the Registrar General and based on the deaths of 1910–12

English Life Table No 11 Based on the deaths of 1950–52 and prepared by the Government Actuary

The results

The basic results of this analysis are

SENESCENT DEATHS

Period of deaths	*Males*			*Females*		
	Peak age	*Standard deviation of distribution*	*Proportion of total deaths*	*Peak age*	*Standard deviation of distribution*	*Proportion of total deaths*
	years	years	%	years	years	%
1841	72.0	9.38	39.0	73.5	9.19	41.0
1910–12	7[illegible].5	8.70	51.5	76.0	8.51	5[illegible].3
1950–52	75.[illegible]	7.89	69.4	80.3	7.12	[illegible]0.3

It is clear that from a practical point of view Clarke was right The main change in the hundred years or so has been the increase in the proportion of people attaining their allotted term of lifespan, from about 40 per cent to 70 per cent, while the shift in the peak has been a mere three or four years for men and seven years for women Judged by the standard deviation the spread of the distribution has narrowed a little but this is not a substantial change If—and it is a large question mark—this arbitrary distribution of "senescent" deaths can be used as an indicator of the natural

distribution of lifespan in humans then it appears possible that over and above the large increase in ability to attain the allotted span, the modal span itself is slowly increasing Even in 1841 there had been a "break through" of the barrier of three score and ten Women can now talk modestly of "four scores

Comparison between men and women

If the proportion of all deaths which are senescent can be used as a measure of ability to survive the allotted term of life, then the improvement in the mortality of women as compared with that of men appears more as a greater shift in the optimum than as a greater improvement in attainment The advantage of women over men (or conversely the dis advantage of men as compared with women) is thus a very general one and calls for intense examination

Other aspects for examination

It would be possible to forecast mortality not only by examining the medical and environmental possibilities for the reduction of anticipated deaths as Clarke suggested but also by predicting the further change in the parameters shown in the table It is tempting for example to suggest that the national life table in 1970–72 will show for men a modal span of almost 77 and an attainment of natural senescence by 78 per cent, but this kind of temptation will be pursued else where

Another possibility is that the attainment" proportion (i e ratio of senescent deaths to all deaths) might be used as a mortality index for international comparison This also is outside the present discussion

The limitations of the life-table

The life table as normally computed is based on the rates of mortality experienced by the population of all ages as they

meniscus from minute to minute as urine collects in it (Hoy and Adolph, 1956). Quantitative collections can also be made without the cannula, at the urinary papilla or by bladder puncture; during rapid urine flows these collections give the same results as with the cannula (Heller, 1947; McCance and Wilkinson, 1947; Falk, 1955).

Water excess, administered by stomach tube, gives rise to very little diuresis at birth (Fig. 4). In the course of several

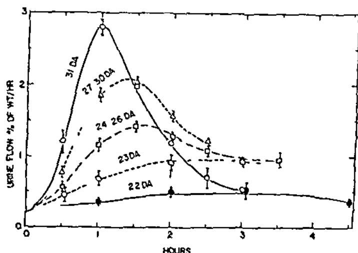


FIG 4 Water diuresis at various ages in infant rats Points show mean and standard error at end of each period of urine collection DA = days after conception From Falk (1955)

days the rat's response increases, until at about ten days after birth the response per unit of body weight is of adult size. The ages indicated on the graphs shown here are reckoned from conception instead of from birth, the average gestation time for rats being 21 3 days Actually in human infants the maturation of the diuresis was found by Ames (1953) to be triggered by birth rather than by scheduled age, since prematures acquired the diuretic response about as soon after birth as postmatures did.

A familiar notion about the way in which water diuresis is excited is to suppose that the neurohypophysis withholds its

diuresis that is large and sudden even a few hours after birth. Likewise adrenaline or noradrenaline induces a full blown diuresis on the very day of birth. Evidently the capacity for excreting water at a high rate is present but its arousal depends on the particular form of stimulation. Consequently any discussion of structural inadequacies or functional immaturities seems beside the present main point, which is that the specific responding system of the newborn rat is not tuned to water excesses.

Hence we are privileged to see a physiological regulation increase in intensity in the growing individual. The regulation

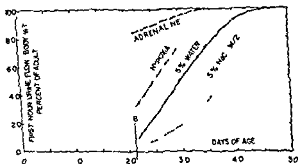


Fig 7 Courses of development of four types of diuresis in rats. B = birth. From Hoy and Adolph (1956)

duly materializes whether the rat has ever experienced a water excess or not; the elements necessary for it are there, some of them long before this materialization. What guides the regulation's intensity and determines its point of adult fixation is unknown. The fixation is still subject to a small degree of adaptation resulting from previous exposure to water excesses (Adolph 1956).

The control of water intake, on the other hand, is much less understood than the control of water elimination. In early infancy rats, like dogs (Adolph 1943 p 267), refuse to drink water even after dehydration. According to Křeček, Krečková and Dlouhá (1956) as late as 28 days after birth young

response is thus uncovered which is mediated through some other channel ordinarily masked by the known hormonal one

The intensity of diuresis is a function of the water excess at all ages (Fig 6), but the regression differs with age Actually these data supply part of an equilibration diagram for infant rats, and by it one can watch the regulatory relations coming to maturity during early postnatal life The unexcreted water has been located as excess in plasma and several other tissues A possible theory of maturation is that some slowly developing process or structure limits the rate of water excretion

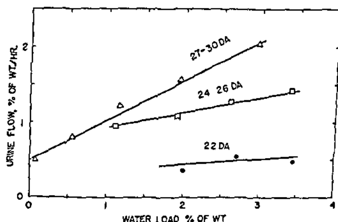


FIG 6 Water exchange in urine in relation to body water load at each of three different ages DA days after conception From Adolph (1957)

This theory is doubtful, since at every age still greater water excess arouses faster excretion Rather, the response, expressed by the ratio between excretory rate and water load is small at birth and becomes greater as age increases

However, in order to see whether diuresis is impossible at birth, we tested the capacity of the infant rat to respond to several other stimuli of diuresis To concentrated salt solutions the diuretic response is practically nil at birth, and it matures even later than the water diuresis (Fig 7) Hypoxia arouses a primary diuresis that is small at birth and becomes greater a few days later, it also, however, arouses a secondary

both absolute (body size) and relative to body solids varies with the age of the rat (Fig 8) What controls the absolute content of water and of each solute? The answer to this question is not available Obviously all the items that enter the determination of growth and its correlatives participate in these controls This is a problem that has barely been visualized and one whose analysis may occupy many physiologists in the future

In general the ready corrections of water excesses and deficits result from specific response systems for diuresis and for water drinking The systems vary between infant and adult, not only quantitatively but possibly also in the mediators and effectors used Over a long lifetime the regulation depends also upon detectors of body size and proportions whose characteristics and locations have not been determined

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DISCUSSION

Shock We have obtained some data in our laboratory on the age differences in the antidiuretic response to pitressin Some of the results of these experiments are in accord with the concept that in many instances the senescent animal returns to a type of response and behavior that is seen during the co...
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rats drink more milk than water in recovering from dehydration But in the same circumstance they drink more water than saline Even newborn rats distinguish between milk and other fluids, at 17 postnatal days they distinguish between water and salt solutions Such sensory discriminations are necessary before rats can link their intakes to specific deficiencies of bodily constituents The actual tying of water drinking to water deficiency does not certainly occur until

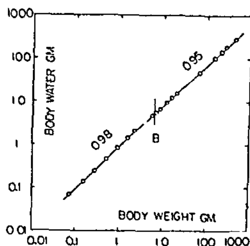


FIG 8 Relation of log water content to log body weight in rats from foetus to adult B = birth Numbers represent exponents in parabolic equation relating the two quantities Data of Hamilton and Dewar (1938) from Adolph (1957)

28 days after birth (Křeček Křečková and Dlouhá, 1956) Already then the water intake of rats equals the water deficit imposed upon them (Adolph Barker and Hoy, 1954 fig 13) just as in the adults, the one hour intake closely matches the water deficit so long as the water deficit does not exceed six per cent of the body weight

Once the immediate regulations of water content are fixed the adult method of maintaining water balance is persistently at work But it is well recognized that the water content,

both absolute (body size) and relative to body solids varies with the age of the rat (Fig 8) What controls the absolute content of water and of each solute? The answer to this question is not available Obviously all the items that enter the determination of growth and its correlatives participate in these controls This is a problem that has barely been visualized and one whose analysis may occupy many physiologists in the future

In general the ready corrections of water excesses and deficits result from specific response systems for diuresis and for water drinking The systems vary between infant and adult not only quantitatively but possibly also in the mediators and effectors used Over a long lifetime the regulation depends also upon detectors of body size and proportions whose characteristics and locations have not been determined

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DISCUSSION

Shock We have obtained some d

is seen durr
measured the co
flows Total unr
subjects the nur

lower total urine output Our results are expressed in terms of the amount of water reabsorbed from the glomerular filtrate, that is the urine/plasma (U/P) ratio of inulin A maximum water diuresis was induced by the oral administration of water plus an intravenous infusion of 5 per cent gluc

old

aged

from 66–90 Under conditions of maximum diuresis the U/P ratio was

esis was again re established in all three groups (Miller J H , and Shock, N W (1953) *J Geront*, 8, 446) (see Shock, Fig 10, p 240)

Heller In connexion with your results, Prof Adolph I should like to clear up a point which has led to some misunderstanding Some years [illegible]

U/P ratios, i e by the same technique as that used by Dr Shock in man, at what postnatal age the antidiuretic response to vasopressin became quantitatively comparable to the response of adult animals We found that this occurred only in rats older than 22 days I would like to stress this because some workers have misinterpreted these results they assumed that we had tried to show that a significant inhibition occurred *for the first time* after 22 days I think that one must expect that this datum of around 20 days may change somewhat in the hands of *other workers Clearly a comparison between the antidiuretic responses* of adult and infant rats depends on the choice and strictness of appli

parable to that in adult animals She found that this occurred at about 17–22 days after birth

Adolph I think Dr Falk (1955) got a significant inhibition considerably before 17 days She also injected vasopressin itself, and by the method of collecting the urine which is expelled in response to perineal

'erstanding to which I have been

to nicotine in animals three days after birth so you are quite right in saying that responses were obtained much earlier *than after 20 days of postnatal life* But she also compared

the response of older animals with that of adults: they became comparable in quantitative terms only when the rats were 17–22 days old.

There is another point on which I should like to have your views, Prof. Adolph. We find that these responses of infant rats to vasopressin are [illegible]

larger animals.

Black [illegible]

[illegible] substances. At the early ages these seem to have very little effect on water diuresis and water excretion.

Saxer I cannot speak about the [illegible]

Heller We have [illegible]

Adolph I should like to make a small protest against the [illegible] term [illegible]

[illegible] immaturity. For [illegible] phosphorus homeo[illegible] functionally imma[illegible] at birth [illegible] is at least [illegible]

[illegible] capacity to [illegible] adrenaline induced

diuresis? Did it increase the ratio of water to solutes in the urine, or did it increase the solute output?

Adolph Adrenaline diuresis in infant rats does involve more solute output than the water diuresis, but adrenaline diuresis is a water diuresis in that the urine is very dilute I do not think you could blame all the adrenaline diuresis on the solute output itself

With regard to immaturity and whether it takes experience for an animal to have a diuresis, we can point to the fact that adrenaline diuresis has no experience factor We have tried to see whether we could get more water diuresis in the infant animal by subjecting it to water loads on successive days There is a considerable variation in the amount of *water excretion which is produced, and we are unable to say that there* is any significant change due to previous experience with water Our provisional conclusion is that there is no adaptation apparent in the animal subjected to repeated water loading

CELLULAR ASPECTS OF THE ELECTROLYTES AND WATER IN BODY FLUIDS

Hugh Davson

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The water and electrolyte contents of a complex organism are almost entirely determined by the activities of the kidneys which operate primarily on the blood plasma and through that on the extracellular fluid of the organism Casual fluctuations in the water and electrolyte contents of the organism are therefore usually the consequence of fluctuations in the composition of these two compartments of the body plasma and extracellular fluid The electrolytes and water of the cells of the body are affected secondarily to these primary fluctuations in the composition of the extracellular fluid and plasma and for practical purposes at any rate the factors that can influence them primarily are usually ignored Nevertheless since the cells occupy a considerable fraction of the total volume of the organism and since there must be some reciprocity between the electrolyte and water content of cells and extracellular fluid it is of some importance that we understand the physical and chemical factors that determine the electrolyte concentrations and volumes of the cells of the body

The Gibbs Donnan Equilibrium The application of the Gibbs Donnan equilibrium to the problem of the water and electrolyte distribution between the plasma and extracellular fluid is familiar to all who have concerned themselves with the water balance of the organism It will be recalled that the most important consequence of the Gibbs Donnan distribution of ions between the two fluids separated by the capillary membrane that is supposed to be impermeable to the protein molecules of plasma is that the osmolarity of the plasma is

significantly higher than that of the extracellular fluid. This is illustrated by Fig. 1, and it follows that an equilibrium will only be achieved when a counter-pressure is exerted on the plasma equal to the colloid osmotic pressure due to the plasma proteins. The amount of this difference of osmotic pressure is determined by the concentration and degree of dissociation of the proteins. Because of the high molecular weights of the plasma proteins, their concentration, expressed as moles per litre, is small and the difference of osmotic pressure that must be resisted, if the system is to remain stable, is correspondingly small, namely 25 mm. Hg. As a result, the organism is able to maintain a statistical equilibrium between plasma and extracellular fluid by virtue of the capillary pressure; at the

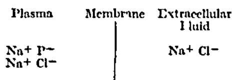


FIG. 1. The plasma-extracellular fluid system (P=protein)

arterial end of the capillary the pressure is greater than this difference of osmotic pressure so that fluid flows into the extracellular compartment, at the venous end the reverse holds, and fluid is absorbed.

It is worth noting that by the term "impermeability" to a solute—here the plasma proteins—we do not necessarily mean an absolute barrier; this is an ideal case on which calculations are based, but practically it seems unlikely that a natural membrane is completely impermeable *to any of the* naturally occurring molecules in solution in the fluids, and it is sufficient for our purposes if by "impermeability" is meant *that the rate of transport* of this solute across the membrane is negligibly small compared with that of the other molecules that we are considering—in the particular case of plasma and extracellular fluid, the salts and water.

The cell membrane is a more selective barrier than the

capillary endothelium, and is capable of imposing restrictions on the movements of ions that are very much smaller than the protein ions, as a result, it is conceivable that much larger differences of osmotic pressure could be established, since these smaller ions may be present in vastly higher concentrations than those of proteins with their large molecular weights Let us consider the erythrocyte, for simplicity we may choose the cat or dog erythrocyte which shows no accumulation of potassium The distribution of ions is indicated roughly in Fig 2, the cell contains the protein haemoglobin which behaves as an anion, so that we may expect to be able to apply the Gibbs Donnan equilibrium to the diffusible ions If the Na^+, Cl^- and HCO_3^- ions could diffuse across the membrane, the position would be entirely



FIG 2 The cat erythrocyte (Hb=haemoglobin)

analogous with that already considered, and the contents of the cell [illegible]
plasma [illegible]
sion cau [illegible]
and swe [illegible] since this difference of osmolarity must prevail so long as the cell contains a higher protein concentration than that in the outside medium Cell membranes are not strong and would certainly not be able to resist the difference of osmotic pressure that would be developed, which in this case would be several times higher than in the case considered earlier owing to the very high concentration of protein in the red cell We know that the cat erythrocyte is stable and we must ask how? Theoretically, stability could be achieved by making the membrane impermeable to salt i e to all the [illegible]
be [illegible]

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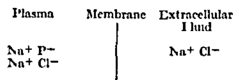


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The cell membrane is a more selective barrier than the

two main factors—the osmolarity of the plasma and the activity of this Na^+ extrusion mechanism The passage of water across the cell membrane is very rapid, so that the cell responds to changes in osmolarity of the plasma by virtually instantaneous changes in its water content, in this way it may be said to respond passively to changes in the plasma, and its changes of water content and salt concentration may be said to be secondary to primary changes determined principally by the kidney The operation of the second factor —the Na^+ extrusion mechanism—will influence the amount of material—salts and water—in the cell, and it would be by virtue of this mechanism that this type of cell could exert a primary influence on the water and electrolyte content of the organism Thus, if the Na^+ extrusion mechanism operated more rapidly than the influx under the electrochemical gradient there would be a net loss of Na^+ and of anions, namely Cl^- and HCO_3^-, this would decrease the osmolarity of the cell and water would be lost to the plasma Such a shrinkage of cells is easily demonstrable by allowing them to recover from the effects of putting the Na^+ extrusion mechanism out of action Thus when the cells are cooled, the metabolic processes supplying energy can no longer work, Na^+ enters the cells accompanied by anions and they swell When the cells are warmed the metabolic processes begin, and the extra Na^+ is excreted until the cells return to their normal volume The effects of agents that increase the permeability of the cell membrane are of some interest, substances like alcohol or urethane in the appropriate concentration, can increase the permeability of the cell membrane to Na^+ and K^+ to such an extent that the Na^+ extrusion mechanism is unable to keep pace with the influx of this ion, thus, in spite of a normally functioning metabolism the cell may swell, on removing the agent it may return to its normal size

The erythrocytes of most species contain K^+ as their principal cation so that the cell maintains large gradients of Na^+ and K^+ (Fig 8) The condition for an osmotically stable system could be given by an impermeability of cations,

impermeable to cations such as Na^+ and K^+ In this way the cell would be able to fulfil its function in the maintenance of the acid base balance of the body, permitting the Cl^-—HCO_3^- exchange that mediates the buffer action of haemoglobin in the cell

[illegible]

membrane, so that any Donnan effect due to impermeable cations on one side of the membrane will be counterbalanced by an equal effect due to impermeable ions on the other side

It is easy to show that an osmotic equilibrium between the inside and outside of the cell is possible, in spite of the high concentration of indiffusible protein anions within the cell, thus the impermeability of the cell membrane to cations such as Na^+ confers on it a stability that would be lacking in the presence of a permeability to this ion, in other words, the colloid osmotic pressure of the cellular proteins can only operate in the presence of a permeability to both Na^+ and anions It is now well known, however, that cell membranes do not show an absolute impermeability to such ions as Na^+ or K^+, the use of isotopes has permitted the demonstration of an unequivocal exchange of these ions across the erythrocyte membrane The exchanges are very slow compared with the exchanges of Cl^- and HCO_3^-, *but they do occur* so that we must expect a constant movement of NaCl and $NaHCO_3$ into the cell, associated with the migration of water unless some process prevents this As is well known the process that does prevent it is an active transport of Na^+ ions out of the cell the membrane is permeable to Na^+ so that there is a continual drift of this ion into the cell because of the demands of the Gibbs Donnan distribution, but by some process not understood, metabolic *energy of the cell is employed in driving the* salt out Practically, in consequence, the cell may be described as a cell impermeable to Na^+ and therefore in stable *equilibrium* with its environment The total amounts of water and electrolytes within the cell will be determined by

excretion of Na^+. The mechanism is not known; presumably the active transport processes are sensitive to the concentrations of Na^+ and K^+, or more probably to the relative proportions of these ions, in the cell

The erythrocyte is a highly specialized cell, and it would not be correct to assume that all cells of the body, or even the majority, are based on a similar physiological plan so far as the maintenance of salt and water content is concerned The striated muscle fibre has been studied very thoroughly, and it may well be that this is far nearer to being a "typical cell", so that we may now consider its main features from the present point of view The main point of difference between the muscle cell and the erythrocyte lies in the low contents of Cl^- and HCO_3^-, these anions being replaced by organic anions

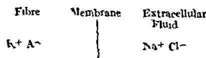


FIG 4 The muscle fibre.
(A^- = indiffusible organic anions)

that apparently cannot diffuse across the plasma membrane, schematically the situation is as in Fig 4 where A^- represents these indiffusible anions The system would be osmotically stable were the membrane impermeable to Na^+, i e the rest of the ions, K^+, Cl^-, HCO_3^-, would distribute themselves across the membrane in such a way that equal osmotic activities would exist on both sides. Actually the cell membrane is permeable to Na^+, and the reason why the Na^+, K^+ and Cl^- ions do not redistribute themselves is because an active extrusion of Na^+, as fast as it penetrates, maintains an effective impermeability to Na^+ There is no need to postulate an active accumulation of K^+ in this case since, owing to the high concentration of impermeable anions in the cell, the extrusion of a Na^+ ion must be associated with the penetration of a K^+ ion in the interests of electrical neutrality Once again, then, the cell may maintain equilibrium with its

as before, but once again studies with isotopes have shown that both Na^+ and K^+ can pass across the membrane and an active transport of Na^+ out of the cell and of K^+ into the cell must be postulated to account for the osmotic stability of the system

It was considered at one time that a mere extrusion of Na^+ would account for the osmotic stability and high concentration of K^+ in the cell, i e that the extrusion of Na^+ would demand a replacement by K^+ It was pointed out however (Davson 1951) that this would lead simply to an excretion of NaCl and $NaHCO_3$ from the cell with a resultant shrinkage Extrusion of Na^+ will only lead to accumulation of K^+ if *exchange of K^+ for Na^+ is obligatory on the system in order to preserve electrical neutrality* If anions can accompany the excreted Na^+ then exchange for K^+ is not obligatory In nerve and muscle where the concentration of non permeable anions in the cell is very high such a sodium excreting mechanism would cause accumulation of K^+

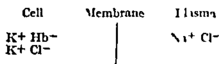


FIG 3 The human erythrocyte (Hb=haemoglobin)

Once again, the water content of such a system will be determined by the osmolarity of the plasma and the activity of the metabolic ionic pumps, thus over activity of the Na^+ excreting mechanism would lead to a shrinkage over activity of the K^+ accumulating mechanism would lead to a swelling It is interesting that the two processes show some degree of linkage, in that Harris (1954) has shown that accumulation of the one ion is associated with a nearly equivalent excretion of the other the linkage is not complete, however, since on cooling erythrocytes swell as a result of gaining more Na^+ than they lose K^+, when they are re warmed the extra Na^+ is excreted and they return to their original volume The fact that the cell maintains its characteristic water content and proportions of Na^+ to K^+ within fairly narrow limits indicates that there is some homeostatic mechanism controlling the rates of accumulation of K^+ and

excretion of Na^+ The mechanism is not known, presumably the active transport processes are sensitive to the concentrations of Na^+ and K^+, or more probably to the relative proportions of these ions, in the cell

The erythrocyte is a highly specialized cell, and it would not be correct to assume that all cells of the body, or even the majority, are based on a similar physiological plan so far as the maintenance of salt and water content is concerned The striated muscle fibre has been studied very thoroughly, and it may well be that this is far nearer to being a 'typical cell', so that we may now consider its main features from the present point of view The main point of difference between the muscle cell and the erythrocyte lies in the low contents of Cl and HCO_3 these anions being replaced by organic anions

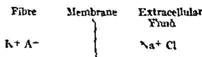


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impermeable anions in the cell, the extrusion of a Na^+ ion must be associated with the penetration of a K^+ ion in the interests of electrical neutrality Once again then the cell may maintain equilibrium with its

environment, provided that an ion excreting mechanism is active. Loss of this, by cooling the tissue or by metabolic poisons, causes a loss of K^+ and a gain of Na^+, Cl^- and HCO_3^-, the net effect being an increase in osmolarity with a consequent swelling of the cells. Re-warming of the tissue may cause a reversal of these changes (see, for example, Steinbach, 1954).

Thus, in all of the cell types that we have considered the system can be treated, theoretically at least, as a system that maintains an osmotic equilibrium between the interior and external fluids by virtue of an 'effective impermeability' to one or more ionic types; if the membrane were truly impermeable to the ions in question, the osmotic equilibrium would be independent of metabolic processes and could be described as a true equilibrium; in practice, the effective impermeability is the result of a continuous process of active transport. For the purposes of mathematical description this is equivalent to an impermeability, at any rate under normal conditions; under abnormal conditions, on the other hand, the precariousness or instability of the equilibrium is shown by the cellular oedema that follows either the failure of the ion excreting mechanism or such a large increase in the permeability of the membrane that the mechanism can no longer keep pace with the influx of Na^+.

If these considerations are correct, we may expect to find that by adding up the total osmolarities inside and outside the cell the two totals should be equal within the limits of experimental error. Probably the muscle fibre has been studied most carefully from this aspect and it would seem from Conway's (1957) figures (Table I) that osmotic equilibrium does exist between the cell and its environment. The same is probably true of the erythrocyte and the nerve fibre, but it must be remembered that the analytical techniques for all the constituents of the cell are not so accurate that a difference of one or two per cent would be ascertained. Within this limit, then, it seems quite safe to affirm that these cells are in osmotic equilibrium with their environment.

Within recent years the possibility that mammalian cells

are not in osmotic equilibrium with their extracellular fluid has been seriously maintained, and an "osmotic pump", driving water continuously out of the cell, has been postulated The experimental basis for this claim rests on the observation that mammalian tissue slices, in particular those of liver and kidney, swell when placed in "isotonic" solutions of sodium chloride, Tyrode or Krebs (Sperry and

Table I

COMPOSITION OF FROG MUSCLE AND PLASMA EXPRESSED AS M MOLE PER kg H_2O (after Conway, 1957)

	Fibre Concentration	*Plasma Concentration*
K	124	2 25
Na	10 4 (3 6)*	109
Ca	4 0	2 1
Mg	14 0	1 25
Cl	1 5	77 5
HCO_3	12 4	26 6
Phosphate	7 3	3 3
Sulphate	0 4	2 0
Phosphocreatine	35 2	—
Carnosine	14 7	—
NH_2-acids	8 8	7 2
Creatine	7 4	2 2
Lactate	3 9	3 5
Adenosine triphosphate	4 0	—
Hexose monophosphate	2 5	—
Glucose	—	4 1
Protein	0 6	2 2
Urea	2 0	2 1
Total	248 2	245 3

* Figure in brackets for sodium represents, according to Conway the true intracellular concentration

Brand, 1939, Opie, 1949), either at room temperature or at 0° Robinson (1952) observed that the swelling could be prevented or reversed by maintaining the tissue at 37°, he found also that the swelling occurred in the presence of cyanide at this temperature Since swelling was prevented by using strongly hypertonic solutions—0 55–0 60 M—he concluded that the cells were iso osmotic with these It will be quite clear from what has been said earlier that these facts may be explained just as easily on the assumption that the electrolyte

excreting system fails at low temperature or in the presence of cyanide Thus soaking a muscle at 0° certainly leads to swelling but this is completely accounted for by the gain of Na^+ and Cl^-, warming the muscle causes an excretion of these ions and it returns to its original volume The same argument will apply to other tissues and conclusive proof that this is the principal explanation for the changes taking place on cooling was provided by the elegant experiments of Deyrup (1953) who showed that if the tissues were bathed in iso osmotic sucrose (0 3 M) they failed to swell If the swelling in Ringer solution had been due to a failure of a water excreting mechanism substitution of salt for sucrose should have had no effect, whereas if the swelling had been due primarily to a penetration of NaCl substitution of a non penetrating substance like sucrose would have prevented it It seems safe to conclude then that very large differences of osmolarity between cell contents and their environment such as those postulated by Opie (1949) and Robinson (1952) do not occur The detection of smaller differences that would demand a water pump continuously excreting water from the cell to maintain an osmotic steady state between cells and their environment, must rely on very precise measurements of osmolarity

The depression of freezing point has been employed by a number of workers with a view mainly to testing the claim that mammalian tissues were hypertonic to plasma (Conway and McCormack 1953 *Opie 1954 Brodsky et al 1953 1956* Conway Geoghegan and McCormack 1955 Itoh and Schwartz 1956) but as Conway s studies indicate the interpretation of the results is not easy since an excised tissue when ground up at 0°, *undergoes autolytic changes—in particular the* breakdown of adenosine triphosphate to inosinic acid ammonia and phosphate—that lead to a considerable increase in osmolarity It would seem from Conway s studies that within the limits of accuracy of the cryoscopic method—probably a few per cent—the tissue cells examined liver kidney and muscle—are iso osmotic with their environment

This does not mean, however, that the maintenance of differences of osmotic pressure between cells and their environment by the excretion of water does not occur, it is well known that such fluids as urine and saliva have osmolarities that are vastly different from that of the plasma, and the elaboration of these fluids is best described by invoking an active transport of water—i e the functioning of a "water

Table II

CONCENTRATIONS OF IONS (M MOLE/kg H_2O) IN PLASMA AQUEOUS HUMOUR AND CEREBROSPINAL FLUID OF THE RABBIT

Plasma				*Aqueous Humour*			
Na	151 5	Cl	108	Na	143 5	Cl	109 5
K	5 5	HCO_3	27 4	K	5 5	HCO_3	33 6
Ca	3 6	Lactate	7 9	Ca	2 3	Lactate	6 00
Mg	1 0	Phosphate	1 8	Mg	0 85	Phosphate	1 00
						Ascorbate	1 00
Total	160 6	Total	145 1	Total	152 1	Total	151 1

Cations and Anions 305 7 — *Cations and Anions* 303 3

Cerebrospinal Fluid

Na	151	Cl	129
K	3 5	HCO_3	31 4
Ca	1 3	Lactate	2 6
Mg	0 8	Phosphate	0 5
Total	156 6	Total	163 5

Cations and Anions 320 1

pump' The cerebrospinal fluid would appear to represent another example of a non iso-osmotic fluid, and since it is in such close relationship with the nervous tissue of the brain and spinal cord, this lack of iso osmolarity is of special interest, suggesting as it does that these tissues, too, are not in osmotic equilibrium with the blood The results of a detailed analysis of the ionic concentrations in plasma and cerebrospinal fluid are shown in Table II, included are values for a similar

excreting system fails at low temperature or in the presence of cyanide. Thus, soaking a muscle at 0° certainly leads to swelling, but this is completely accounted for by the gain of Na^+ and Cl^-; warming the muscle causes an excretion of these ions and it returns to its original volume. The same argument will apply to other tissues, and conclusive proof that this is the principal explanation for the changes taking place on cooling was provided by the elegant experiments of Deyrup (1953) who showed that if the tissues were bathed in iso-osmotic sucrose (0·3 M) they failed to swell. If the swelling in Ringer solution had been due to a failure of a water-excreting mechanism, substitution of salt for sucrose should have had no effect, whereas if the swelling had been due primarily to a penetration of NaCl, substitution of a non-penetrating substance like sucrose would have prevented it. It seems safe to conclude, then, that very large differences of osmolarity between cell contents and their environment, such as those postulated by Opie (1949) and Robinson (1952), do not occur. The detection of smaller differences, that would demand a water pump continuously excreting water from the cell to maintain an osmotic steady state between cells and their environment, must rely on very precise measurements of osmolarity.

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This does not mean, however, that the maintenance of differences of osmotic pressure between cells and their environment by the excretion of water does not occur, it is well known that such fluids as urine and saliva have osmolarities that are vastly different from that of the plasma; and the elaboration of these fluids is best described by invoking an active transport of water—i e the functioning of a "water pump" The cerebrospinal fluid would appear to represent another example of a non iso-osmotic fluid, and since it is in such close relationship with the nervous tissue of the brain and spinal cord, this lack of iso osmolarity is of special interest, suggesting as it does that these tissues, too, are not in osmotic equilibrium with the blood The results of a detailed analysis of the ionic concentrations in plasma and cerebrospinal fluid are shown in Table II, included are values for a similar

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CONCENTRATIONS OF IONS (M MOLE/kg H_2O) IN PLASMA AQUEOUS HUMOUR AND CEREBROSPINAL FLUID OF THE RABBIT

Plasma				*Aqueous Humour*			
Na	151.5	Cl	108	Na	143.5	Cl	109.5
K	5.5	HCO_3	27.4	K	5.5	HCO_3	33.6
Ca	2.6	Lactate	7.9	Ca	2.3	Lactate	6.00
Mg	1.0	Phosphate	1.8	Mg	0.85	Phosphate	1.00
						Ascorbate	1.00
Total	160.6	Total	145.1	Total	132.1	Total	151.1

Cations and Anions 305.7 *Cations and Anions* 303.3

Cerebrospinal Fluid			
Na	151	Cl	129
K	3.5	HCO_3	31.4
Ca	1.3	Lactate	2.6
Mg	0.8	Phosphate	0.5
Total	156.6	Total	163.5

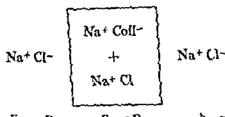
Cations and Anions 320.1

type of fluid, the aqueous humour—similar because both are specialized tissue fluids filling cavities and being virtually free from protein By summing the cations and anions it becomes clear that the cerebrospinal fluid has a higher concentration than the plasma or the aqueous humour, allowance must be made for the lower concentrations of glucose and urea in the cerebrospinal fluid, a difference amounting to some 5 m mole, thus the cerebrospinal fluid is hyperosmotic by some 9 m mole The amount is small—some 3 per cent—nevertheless it represents a difference of osmotic pressure of some 160 mm Hg, and it is presumably because the fluid is able to drain away easily from its cavities that this pressure does not develop, i e the difference in osmolarity is reflected in a continuous influx of water from the blood rather than in the development of a pressure, such as would happen were the system completely closed However, the really significant point to be made in this connexion is that the cerebrospinal fluid lies in such close relationship with the brain and cord that it seems most unlikely, having regard to the rapidity with which water may exchange between the two, that a difference of osmolarity could be maintained That is, if the cerebrospinal fluid is, indeed, hypertonic to plasma, then so must the tissue of the brain and cord be If this is true, then we may postulate one of two things either a water pump that drives water out of the nerve cells into the extracellular fluid where it passes back into the blood, or alternatively the elaboration, by the capillaries of the nervous tissue, of a hyperosmotic extracellular fluid The capillaries in this region of the body are certainly different from those in the rest of the body and are responsible, presumably, for the so called "blood brain barrier", to attribute secretory activity to their endothelium is by no means an unreasonable proposition The important point to be made here is that the difference of osmolarity is small and thus requires highly accurate analysis for its demonstration Why the cerebrospinal fluid and nervous tissue should have this higher osmolarity is not clear, according to Flexner (1938),

the high concentration of chloride in the cerebrospinal fluid,

in fact It may be that the positive p
spinal fluid depends for its maintenance on a difference of osmotic pressure between it and the blood

The factors determining the water and electrolyte contents of connective tissue are probably simple, although they have not been studied in great detail If a piece of collagen, or collagen plus mucoid, is placed in a saline medium, equivalent to extracellular fluid, we may expect a Gibbs Donnan equilibrium to be established between this and the medium by



phase

virtue of the *acidic* nature of the protein and mucoid The situation *might therefore be as in Fig 5, i e* essentially similar to that obtaining with plasma separated by a membrane from *extracellular fluid* There is no membrane separating the *two* however and separation is maintained because of a phase *difference the* collagen mucoid system being a gel, *the extracellular fluid* a *liquid Chemical* analysis of connective tissue shows that there is indeed, a Gibbs Donnan distribution of ions between it and plasma and therefore, presumably between it *and* extracellular fluid, the concentration of chloride *being less* and that of sodium greater, in the *connective tissue There is* in consequence, a tendency for water to pass into the connective tissue phase, the salts

continuously redistributing themselves so that the osmotic pressure of this phase is greater than that of extracellular fluid and of blood The extent to which the system will take up water will depend on the counter pressure that can be exerted or, failing that, what is really equivalent, the mechanical rigidity of the system that will oppose distention Presumably in such tissues as tendon and skin the structural rigidity of the system prevents an indefinite uptake of water, and the system is stabilized with a water content of about 75 per cent In the cornea of the eye, however, the situation

Table III

COMPARISON OF EYES MAINTAINED AT NORMAL AND LOW CORNEAL TEMPERATURES

(Davson, 1955)

Expt no	Temp (°)	Time (hr)	Water Content (g /100 g tissue)	Water Content (g /g solid)
1	7	15	82 8	4 8
	31	—	77 2	3 4
2	7	15	82 8	4 8
	31	—	77 0	3 35
3	7	17	82 1	4 65
	31	—	78 2	3 6
4	7	7	78 5	3 65
	31	—	77 8	3 5

is different, it consists, essentially, of a number of laminae of collagen plus mucoid, sandwiched between two cellular layers, the epithelium and endothelium If the eye is excised and stored in the cold, say at 4°, the cornea increases in water content, due to absorption of aqueous humour If instead of being kept at 4° the eye is maintained at about 31°—the normal temperature of the cornea—the tissue retains its normal water content (Table III) It would seem, then, that metabolic activity is preventing the collagen plus mucoid from absorbing water and salts from the aqueous humour, and this may be proved by first allowing the cornea to swell at the

low temperature and then transferring the eye to a chamber maintained at the higher temperature In this case the ab sorbed water and salts are excreted back and the cornea reacquires its normal hydration (Table IV) The secretory activity that usually maintains the cornea in its normal state of hydration—about 75 per cent water—may be due to both the endothelium and epithelium but whether it is due to an active excretion of salt e g sodium or of water remains to be proved The extraordinary tendency of the cornea to take up water by contrast with tendon or sclera, is presumably

Table IV

THE EFFECT OF SUBSEQUENT WARMING ON EYES MAINTAINED FOR 15–18 HOURS AT 7

(Davson 1955)

Column A gives the water content after the period at *° column B the water content after a further period of 6–8 hours at 31°

Expt no	Water content (g/g solid) (A)	(B)	Change (%)
1	4 35	3 3	24
2	5 1	3 0	41
3	4 45	3 7	17
4	4 65	3 9	16

related to the large quantity of mucoid present as a coating over the individual collagen fibrils (Schwarz 1953) and it seems likely that changes in hydration are really the conse quence of changes in hydration of this *colloid* the collagen fibrils being pushed apart by the swelling The Gibbs Donnan swelling of the collagen mucoid system of skin and subcutaneous tissues may well be a factor in determining the water content and the turgescence of the tissues Thus it would seem from *McMaster s (1946)* studies that the extra cellular fluid may *in normal* circumstances be something of an abstraction the space between cells and collagen fibrils being occupied by a mucoid gel only when excessive amounts of fluid are filtered from the plasma or under experimental

conditions of injection of fluid into the tissue, is it possible to speak of free fluid in the extracellular spaces The nature of the collagen and mucoid in these tissues may therefore exert some effect on the water content of the tissues In general it would seem that acute changes in this tissue extracellular water are the result of changed factors of capillary filtration and reabsorption, but it may well be that the long term steady state level is influenced by the amount of mucoid in the tissue This presumably exerts its Gibbs Donnan difference of osmotic pressure, drawing fluid to it, the tendency is opposed by the structural rigidity of the tissue, so that a steady state is established in contrast to the cornea where the rigidity of the system is inadequate to permit a steady state a continuous secretory activity being necessary, and made possible by the presence of cellular membranes lining the tissue

The possible ways in which the water compartments of the body may be altered with age become evident from this general review, thus the activity of the ion transporting mechanisms of the cells tends to oppose a normal tendency to cell oedema, with the result that a steady state is maintained with the cells having a characteristic ionic make up and percentage of water A decrease in the metabolic activity of the cells may be expected to result in the penetration of salt and water into the cells, hyperactivity, on the other hand may cause a shrinkage of the cells but the extent of this will be limited by the demands of electrical neutrality excessive excretion of the Na^+ ion must be associated with excretion of some anion or with accumulation of K^+ in the latter event there will be no change in osmolarity, whilst the former process is limited by the availability of diffusible anions It seems unlikely that a cellular dehydration could result from hyper activity of this sort, and it seems more likely that dehydration of cells might be due to a loss of the indiffusible anions collectively indicated as A^- in Fig 4 but actually consisting of proteins, organic phosphates etc If these were replaced by such diffusible anions as Cl^- and HCO_3^-, then the process of

extrusion of Na^+ would lead to an elimination of these ions and it could well be that a new steady state would be established at a lower level of internal K^+ and Na^+ concentrations Unfortunately, practically nothing is known of the factors that control the normal activity of the salt excreting system of the cell

The large differences in the amount of extracellular water that take place with age may be to some extent, associated with differences in the amount of water per cell of the organism thus other things being equal a decrease in cellular water is reflected in a rise in the extracellular water, expressed as a percentage To prove this however, it would be necessary to measure not so much the percentage water in the cells as the amount of water per cell and this might be attempted by relating the water to the deoxyribonucleic acid content of the tissue It seems more likely however, that long term fluctuations in the fractions of intra and extracellular water, especially those taking place during development will be determined b[illegible]
of tissue rath[illegible]
achieved by[illegible]
cells (b) expansion or contraction of the extracellular space by changes in the quantity of connective tissue and in the ability of this to hold fluid

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representative sample of what is going on in the organism. Is this a fair assumption? A second point is that most of the work has been done on kidney slices Are kidney slices representative of the whole organism, or only of the kidney tissue?

Fejfar Cort and Kleinzeller find that the amount of potassium

Fejfar Roguski in Poland claims that one can judge general cellular metabolism from the red cell

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only enter cells in significant amounts and that only sodium does this, so we judge the extracellular fluid by the chloride present

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DISCUSSION

Talbot I was most interested, Dr. Davson, in your comments about the cellular oedema that occurs in 'sick' cells It has been shown that animals deprived of potassium, and thereby subjected to a combination of cellular potassium insufficiency and cellular sodium intoxication, show a tendency to cellular oedema. We therefore wondered whether loss of potassium from the cell was a factor which might interfere with its sodium and water pump mechanisms.

ment of more of these ions and to a condition in which there is a high potassium concentration inside, and low sodium and chloride. When we allow the system to cool or give it poison, then we find that sodium comes

... we do not really know ... ther ... um.

Davson So you propose a condition where there is a sodium as well as a potassium deficiency?

Talbot You could have simple deprivation with loss of cellular potas- ... ere ... lar ... fit

Davson It is really a matter of thinking these things out as separate

when we analyse a muscle biopsy specimen, that we

Maybe someone can furnish data which will be more convincing on whether the *electrolyte* transfers are equally rapid and reproducible.

Davson I think the electrolyte transfer is very likely to be much slower and to hold up the whole process. The water transfer is very rapid in every cell so I would say that what happens first is the movement of the electrolyte and the movement of water would not require much time The evidence I am citing is largely based on work from Prof. Conway's laboratory

[illegible]

[illegible] and water then entering to preserve osmotic balance (Conway, E J , and Geoghegan, H (1955) J *Physiol* , 130, 438)

Dawson That would be a most dangerous conclusion to draw. If your chloride space altered under experimental conditions, it could very well be due to penetration of chloride into the cells

Wallace We have been working with tissues for some time from the standpoint of hydrogen ion gradients between cells and extracellular

analytical values for whole tissues They point out that the interior of the cell is not homogeneous Potassium and sodium do not appear to be evenly distributed and the hydrogen ion concentration seems to vary from locus to locus I am certainly not ready to give up the study of ions and their distribution in tissues, but I think one must always bear in mind that membrane equilibria can only tell a part of the story The concept of the cell, particularly the muscle cell, as an "empty bag" cannot be completely accepted

Dawson In general I am in favour of your iconoclastic approach but you are basing most of your argument on the findings of the electron microscopists and they are by no means above criticism themselves They are working on fixed tissue and talk about their endoplasmic reticulum It certainly appears as a most complicated system of canals, but one wonders how r

elementary ph

complexities?

brane with certain permeability characteristics The electron microscopists show us the membrane which does exist but then they find little

have obviously got to be suspicious of treating things too simply—there you are absolutely right On the other hand I am not willing to stop applying elementary physical chemistry to problems of salt transfer just because of these complexities

Adolph I should like to add something to the point about swelling and shrinking with the accompanying transfers of electrolytes When tissue slices, not only kidney slices but also liver slices and two tissues which we did not have to slice i e diaphragm and auricle are transferred from

vinced that the electrolyte transfers are necessary for this swelling and shrinking. We have no method of measuring the speed of the electrolyte transfers, but we have a method of measuring that of the water transfers

Before discussing the subject in more detail it may be helpful to recall some of the factors which regulate the water content of the body

Regulation of water

Two mechanisms, closely linked, normally guard against water depletion One regulates the intake of water through the sensation of thirst, the other the output of water through the secretion of antidiuretic hormone There are at least two ways in which each may be invoked the first, a rise in the tonicity the second, less well known, a fall in the volume of the body fluids (Smith, 1957, Strauss, 1957)

A rise in the sodium content of the extracellular fluid (ECF) is well known to produce thirst and to stimulate the release of antidiuretic hormone (ADH) The *effective stimulus* is not simply the rise in ECF tonicity if the ECF tonicity is raised with a substance like urea, which diffuses freely across the cell membrane and raises the tonicity of both extracellular and cellular fluid equally, this does not stimulate thirst and antidiuresis to the same extent (Gilman, 1937) *When*, however, the extracellular tonicity is raised by a *substance* which does not diffuse into the cells, water leaves *the cells* until the tonicity of extracellular fluid and cells are again equal *The cells shrink It is* assumed that certain cells in the [illegible] *stimulate* the sensa [illegible]

[illegible] there is much evidence that there are localized receptors of this kind (Jewell and Verney, 1957, Verney 1957) Recently Andersson (1957) has also provided additional evidence for a thirst centre He found that when he stimulated a *certain area* of the hypothalamus in goats, they drank water as long as *the stimulus* went on, even to the point of haemolysing their own red cells *With* destructive lesions in the same region, the goats would not drink water when they obviously *needed* it The thirst centre and the receptors of the ADH *mechanism* are very close together, but probably distinct

HYPERNATRAEMIA AND HYPONATRAEMIA WITH SPECIAL REFERENCE TO CEREBRAL DISTURBANCES

PAUL FOURMAN and PATRICIA M LEESON

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Introduction

AN *abnormal concentration of sodium in the extracellular* fluid often presents a puzzling problem for the clinician As is well known, a change in the total amount of the sodium or of the water in the body can explain many instances—water deficiency or sodium excess producing hypernatraemia water excess or sodium deficiency producing hyponatraemia But many cases appear to require more than a simple account of gains and losses to explain them Is this because a simple explanation, such as a change in the amount of water in the body, has been overlooked, or must one in such cases invoke some new mechanism, possibly under the control of the nervous system?

There have been a number of reports of 'cerebral" hyper natraemia and hyponatraemia (Knowles, 1956, Edelman, 1956) With regard to hypernatraemia it seems likely that some of the contradictions in the present views (Welt *et al*, 1952, Higgins *et al*, 1954) might have been avoided, for in hardly any of the patients reported could a frank water deficiency confidently be excluded from the information supplied This question is discussed in the first section The *subject of hyponatraemia seems much more difficult but if sodium deficiency is excluded, many of the remaining cases* can be accounted for by an abnormal retention of water diluting the body fluids In the second section we present some new data on the problem, derived from a study of two patients

nexion it may be recalled that two of Allott's patients had had an alimentary haemorrhage

It is partly through neglect of this phenomenon that some authors have been led to place cases of hypernatraemia with a low urinary sodium in a separate group

Symptoms of water deficiency There are several reasons why authors describing neurogenic or cerebral hypernatraemia may have overlooked a water deficiency Though they *often* state that there is no clinical evidence of dehydration in their patients (e g Cooper and Crevier, 1952), this does not in fact mean very much The word *dehydration* is used for two clinical states one of water deficiency alone, and the other of salt deficiency which generally also entails a loss of water This usage implies that these deficiencies produce a similar clinical picture, though it was made clear long ago that this is not so (Kerpel Fronius 1935 Nadal, Pedersen and Maddock, 1941) Water deficiency is not clinically obvious unless it is *extreme*, because the deficit is distributed throughout the body water In salt deficiency, on the other hand the extra cellular fluid though but a third of the total in volume, bears the whole of the deficit, it is patients with the latter who have the haggard look the sunken eyes the small pulse and low blood pressure of dehydration Patients with simple water deficiency are ill but there are no specific signs of the deficiency the tongue may even be moist, and it is not obvious it is water they lack If in addition as a result of a craniotomy their faces are oedematous it may even be mistakenly assumed that they have accumulated water in excess The diagnostic difficulties are increased because, particularly in older patients some of the most striking symptoms of water deficiency are cerebral rather than vascular for instance drowsiness and confusion and disturbances of behaviour, which can mimic a lesion of the frontal lobes These symptoms make it more difficult to give water but they can be completely reversed with water

Losses of water Abnormally large losses of water may go unrecognized Extrarenal losses may be larger than is

The position of these centres in the nervous system suggests that their control involves more than a response to changes in tonicity, and some purely nervous stimuli such as pain and emotion may initiate, or inhibit, thirst or antidiuresis

A fall in the volume of the ECF can stimulate thirst and antidiuresis, presumably through nervous pathways (see Rosenbaum, 1957, Strauss, 1957) Smith (1957) has discussed at length where the receptors for the stimulus to antidiuresis might be some of them may be in the left auricle of the heart (Henry and Pearce, 1956)

Hypernatraemia

Water deficiency

Normally, thirst and antidiuresis are stimulated by a very small increase in extracellular tonicity, less than two per cent (Wolf, 1950, Verney, 1957) A concentration of sodium ([Na]) in the plasma exceeding 150 m equiv /l may certainly be regarded as abnormal In a study of water deficiency produced experimentally in dogs values of 160, and in one animal that died a value of 186 m equiv /l, were found (Elkinton and Taffel, 1942), in a man made water deficient by Black, McCance and Young (1944) the [Na] rose to 160 m equiv /l In a patient from Texas reported by Gordon and Goldner (1957) a value as high as 192 m equiv /l was reported He recovered

The "dehydration reaction" The hypernatraemia of water deficiency is not simply *the result of the blood becoming* more concentrated, for in spite of the high blood level of sodium there may be very little sodium in the urine, it is retained in the body

Allott (1939), who first drew attention to the problem of hypernatraemia, found the urinary [Na] ranged from 2 5 to 9 m equiv /l in four of his patients It now seems most likely these low concentrations of sodium were a result of the "dehydration reaction" first described by Peters (1948, 1952) The mechanism of this reaction is not clear, though it appears to be a renal response to a fall in blood volume, in this con

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generally assumed, and a good urinary output does not necessarily mean there is no deficit of water, for it may represent failure of conservation In the unconscious or helpless patient the intake depends on the physician's instructions and the nurses' care If the intake is less than the combined losses from the skin, the lungs and the bowels, there must be a deficit of water in the body and the plasma [Na] will eventually rise

Some cerebral lesions are associated with a high fever, or with excessive sweating or with an abnormally rapid respiration With any of these the insensible losses of water may increase from the normal value of some 800 ml They have rarely been measured, but in one patient they were thought to be as much as five litres a day (Gordon and Goldner, 1957)

One expects the volume of urine to be small in water deficiency, and its concentration high But there are three ways in which untoward renal losses of water may contribute to water deficiency diabetes insipidus from a failure of the pituitary hypothalamic mechanism, defective renal function, and osmotic diuresis Neither the first nor the second has always been excluded in cases reported as cerebral hypernatraemia Diabetes insipidus possibly explains cases 1 and 3 of Cooper and Crevier (1952) and one case of Natelson and Alexander (1955) The force of this explanation is emphasized by a patient reported by Peters (1948) a young woman whose serum [Na] rose from 140 to 171 m equiv /l in 24 hours following an operation for craniopharyngioma which was complicated by diabetes insipidus In an incontinent patient a low concentration of the urine may be the only clue to diabetes insipidus and the effect of pitressin should be tried in all patients with hypernatraemia in whom this possibility exists

The excretion of a large amount of solutes produces an osmotic diuresis (McCance, 1945, Hervey, McCance and Tayler 1946, Rapoport *et al*, 1949) This happens in spite of a water deficiency (McCance, Young and Black 1944) and may even be the cause of it

Urea, sodium and chloride are the main osmotically active constituents of the urine The excretion of urea may be

increased by an abnormal breakdown of body protein or by excessive protein in the diet. One hundred grams of protein contain 16 g. of nitrogen, excreted as 34 g. or 570 m-osm. of urea. Ten grams of sodium chloride provide 340 m-osm. It is not unusual for unconscious patients to receive these amounts in their feeds; and their endogenous production of urea may already be very large (Cooper *et al.*, 1951). The hypernatraemic patient of Natelson and Alexander (1955) presumably had an osmotic diuresis when he was made worse with "non-saline fluids", because these consisted partly of protein hydrolysate equivalent to 100 g. of protein. In certain neurological disturbances (Astrup, Gotzche and Neukirch, 1954; Whedon and Shorr, 1957) and in water deficiency itself (Black, McCance and Young, 1944) the breakdown of body protein may be greatly accelerated.

To detect a water deficit, the minimum data required are the estimated intake and output of water and solutes, and the volume and concentration of the urine. A water deficit is confirmed if, with the administration of water, the elevated plasma [Na] falls.

In many of the reports of cerebral hypernatraemia it is impossible to decide from the data given what the water balance was. The patients with hypernatraemia of Higgins and his co-workers (1954) seem to have begun with a deficit of water of about one litre. Subsequently their intake of water may have been as little as two litres daily. Their exogenous osmolar load was about 610 *m osm*. We do not know what was the total excretion, urine volumes and specific gravities are not stated. The blood urea was high, and fell as the plasma [Na] fell, when their intake of fluid was increased. In oth[illegible]

[illegible] Thomsen, 1954; Allott, 1957)

Failure of thirst. Even when losses of water do go unrecognized by the clinician, there is no danger of water-depletion as long [illegible]

and is a[illegible]

insipidus the plasma [Na] is not usually very much raised, in a patient of ours, a man of 28 with sarcoidosis, the plasma [Na] was at times as high as 149 m equiv /l, but he was then very thirsty, and he would not tolerate the [Na] rising any higher On the other hand, patients who are apathetic, weak, *disorientated or unconscious may be unaware of thirst,* or unable to respond to it In these patients even normal losses of water may lead to water deficiency with hypernatraemia It is not unusual to have elderly patients with cerebro vascular disease who tolerate a plasma [Na] of 150 m equiv /l without any complaint of thirst But when they are given water they retain it, and their clinical and biochemical responses show they had a need for it We do not know the possible sites of the lesions which may interfere with the sensation of thirst in these people There is, however, some evidence that in man (Leaf and Mamby, 1952, Engstrom and Liebman, 1953), as in the rat (Stevenson, Welt and Orloff, 1950) and the goat (Andersson, 1957), neurological lesions may interfere with the normal sensation of thirst

We have had the opportunity of studying a boy of ten who had had a large suprasellar craniopharyngioma removed by Mr C Langmaid There was no evidence of diabetes insipidus before the operation After the operation however, while he was in a stuporous state, his plasma [Na] ranged between 152 and 163 m equiv /l It remained high even when he recovered, and was up and about, and receiving pitressin The boy did not complain of thirst and we think the lack of thirst led to water deficiency and hypernatraemia These are some of the values before and after he received pitressin —

	Date	*Plasma sodium m equiv /l*	*Urine vol ml per 24 hours*	*Specific gravity*
Before pitressin	7 Nov	161	1370	
After pitressin	21 Nov	150	860	
	25 Nov	156	1420	1 008

The urine volume and specific gravity while he was having pitressin suggest the treatment was inadequate, but he did

not respond, as does the ordinary case of diabetes insipidus, with thirst (He recovered spontaneously from his diabetes insipidus, and from his hypernatraemia, after three months) Although this type of hypernatraemia might be termed cerebral, it is in fact a water deficiency due to the breakdown of one of the mechanisms that normally ensure water balance

Renal effects of water deficiency Before leaving the question of hypernatraemia due to water deficiency it may be noted that in many of the reported cases the disturbance apparently produced a disorder of tubular function, manifested by oliguria with isosthenuria or by the excretion of urine with a high pH in the presence of a systemic acidosis (Cooper and Crevier, 1952 (Case 4), Gordon and Goldner, 1957, Allott, 1957) This suggests that severe water deficiency may be accompanied by tubular damage, Allott (1930) noted a tubular degeneration in two of his cases *post mortem*

A tubular damage wou

least one of the patients

It is not possible to say

patients were water deficient but all of them had a high blood urea and in relation to this the urine volumes were certainly small It is also possible that in some patients (e g Allott 1957) polyuria with hyposthenuria represented the diuretic phase of a tubular *necrosis*, *itself* the result of dehydration

To sum up the question of 'cerebral' hypernatraemia, a failure of the thirst mechanism with or without a diabetes insipidus accounts for some of the cases that have been described and as Gordon and Goldner (1957) have ably illustrated unrecognized renal or extrarenal losses of fluid

e result of

if enough

- restimated

unt of water required to correct a severe deficit Higgins and co workers (1954) gave up to four litres to the patients they thought were water deficient We give nearly

this amount routinely Gordon and Goldner gave one of their two patients 8 24 litres in 24 hours and even this was not enough to bring down his plasma [Na] to normal As long as the plasma [Na] remains high there can be no risk of water intoxication

Other forms of hypernatraemia

It is possible to produce hypernatraemia by giving an excess of salt (McCance, 1956) though more usually this produces an isotonic expansion of the extracellular fluid with oedema

The homeostatic mechanisms may be so adjusted as to *maintain the plasma [Na] at a high level* In experimental potassium deficiency the plasma [Na] was over 150 m equiv /l although the absorption of sodium was small and the intake of water as much as eight litres a day in one subject (Fourman 1954) Hypernatraemia is often a feature of aldosteronism *(Conn 1956) but whether or not this is the result of the* associated potassium deficiency cannot be stated Recently Zilva and Harris Jones (1957) have discussed the possibility of excessive adrenocortical activity producing hypernatraemia by a shift of sodium from cells to ECF

Hyponatraemia

We may arbitrarily define hyponatraemia as a plasma [Na] lower than 130 m equiv /l It is obvious the concentration of sodium in the plasma may fall because of a reduction in the *total amount of sodium in the ECF or because of an increase* in the amount of water

Salt deficiency

A reduction of the *total* amount of sodium in the ECF is *the result of sodium deficiency*

We have already emphasized that the clinical effects of sodium deficiency are easily recognizable Lack of salt is unlikely to arise unless through sweating vomiting diarrhoea or fistulous discharge, sodium is lost from the body because

the kidneys normally conserve sodium efficiently For the same reason in sodium deficiency there is virtually no sodium in the urine To this there is one exception, namely, when the sodium deficit is actually the result of continued loss through the kidney This happens, of course, in Addison's disease, and in 'salt losing nephritis" Furthermore, in certain patients with cerebral lesions persistent renal losses have been observed, even when the intake of sodium is much reduced (Welt *et al* 1952) The renal defect has been ascribed to a loss of neural impulses affecting proximal tubular function (Cort 1954) But the patient of Merrill, Murray and Harrison (1956) with malignant hypertension was able to maintain a normal sodium balance when his own kidneys were replaced by a kidney which was transplanted from his twin brother and therefore deprived of its nerve supply It does not seem then that a *loss* of nervous impulses is alone *responsible* for a failure of the kidneys to conserve salt, though the renal nerves do play a part in the response to salt deprivation (Bricker *et al*, 1956) and to anoxia (Földi, Kovách and Takács 1955*a b*) The mechanism of the defect in cerebral salt wasting remains obscure Water excess (*see* below) may produce a renal loss of sodium and some instances of so called salt wasting may therefore be examples of water retention

Hyponatraemia from salt deficiency can of course, be corrected with salt

wi

at

part of the body produces a relative lack of salt and water If only water is provided the [Na] falls This state of affairs is seen most clearl aft

tion of

for the patient while drinking copiously, produces only a small amount of concentrated urine containing very little sodium (Nelson, Rosenbaum and Strauss, 1951)

Water excess

Water excess is a well recognized cause of hyponatraemia when patients are given too much water while unable to excrete it at the normal rate (Wynn, 1956) This may happen in renal failure, in adrenal and pituitary insufficiency, and postoperatively, particularly after mitral valvotomy (Bruce *et al*, 1955) Hyponatraemia from this cause is usually obvious from the circumstances Such patients may have no symptoms, sometimes they have the syndrome of water intoxication, with *fits and other profound neurological disturbances* They may have hypertension, they certainly do not have hypotension The face looks bloated, not drawn

Both sodium deficiency and simple water excess respond to the administration of hypertonic saline with a rise in the plasma [Na] to normal which is subsequently maintained

There remains for consideration a large group of cases where the hyponatraemia does not produce symptoms and its mechanism is obscure Elkinton (1956) and McCrory and Macaulay (1957) have recently reviewed this problem The hyponatraemia appears to be associated with an expanded volume of ECF, and the kidneys do not excrete water or retain sodium to bring back the tonicity of the plasma to normal (Leaf and Mamby, 1952)

There are at least two possible explanations The first is that there is an abnormal stimulus to antidiuresis, say from the 'volume receptors", operating through the secretion of ADH or in some other way (Kleeman *et al*, 1955, Ginsburg and Brown, 1957) Pitressin given experimentally to normal people leads to a retention of water, a fall in the plasma [Na] and eventually an increased renal loss of sodium in spite of the low plasma [Na] (Leaf *et al*, 1953, Weston *et al*, 1953, Wrong, 1956)

The second possibility is that an abnormal hypotonicity of the cells determines the hypotonicity of the ECF (Sims *et al*, 1950, Rapoport, West and Brodsky, 1951)

McCrory and Macaulay (1957) described an infant with diffuse cerebral damage and hyponatraemia Her ECF

volume was greater than normal The infant did not excrete a dose of water at the normal rate and the authors thought she was secreting an excess of ADH An excessive secretion of ADH would, of course, be appropriate only to a restricted fluid intake When her fluid intake was restricted the plasma [Na] rose to normal

Schwartz and co workers (1957) have recently suggested that an inappropriate secretion of ADH might account for the hyponatraemia in two patients with carcinoma of the bronchus whom they studied They imply that there was an abnormal stimulation of the receptors for maintaining the volume of the body fluids Their patients had normal renal and adrenal function, they excreted a normal amount of aldosterone In one of them the plasma [Na] fell as low as 103 m equiv /l, but the extracellular volume, far from being reduced as in sodium deficiency was expanded and there was no evidence of peripheral vascular failure The urine was generally hypertonic to the plasma, and this is the principal argument adduced by Schwartz and co workers that these patients were producing too much ADH The kidneys of these patients did not conserve sodium when their fluid intake was unrestricted though they did so when large amounts of salt retaining steroids were given Schwartz and co workers de

dose of

normall

urinary … and the plasma [Na] rose Others have also described this response to water deprivation in hypo natraemia (see Edelman, 1956) It might be interpreted as the usual ' dehydration reaction"

Some observations we have made on two patients with unexplained hyponatraemia are relevant

Case reports

Albert aged 62 was admitted on 25th May 1957 in status epilepticus accompanied by hyperpyrexia and heavy sweating He had been up and about until then although he had had a right hemiparesis for two years which had become worse two months before admission His

Water excess

Water excess is a well recognized cause of hyponatraemia when patients are given too much water while unable to excrete it at the normal rate (Wynn, 1956) This may happen in renal failure, in adrenal and pituitary insufficiency, and postoperatively, particularly after mitral valvotomy (Bruce *et al*, 1955) Hyponatraemia from this cause is usually obvious from the circumstances Such patients may have no symptoms, sometimes they have the syndrome of water intoxication, with fits and other profound neurological disturbances They may have hypertension, they certainly do not have hypotension The face looks bloated, not drawn

Both sodium deficiency and simple water excess respond to the administration of hypertonic saline with a rise in the plasma [Na] to normal which is subsequently maintained

There remains for consideration a large group of cases where the hyponatraemia does not produce symptoms and its mechanism is obscure Elkinton (1956) and McCrory and Macaulay (1957) have recently reviewed this problem The hyponatraemia appears to be associated with an expanded volume of ECF, and the kidneys do not excrete water or retain sodium to bring back the tonicity of the plasma to normal (Leaf and Mamby, 1952)

There are at least two possible explanations The first is that there is an abnormal stimulus to antidiuresis, say from the "volume receptors", operating through the secretion of ADH or in some other way (Kleeman *et al*, 1955, Ginsburg and Brown, 1957) Pitressin given experimentally to normal people leads to a retention of water, a fall in the plasma [Na] and eventually an increased renal loss of sodium in spite of the low plasma [Na] (Leaf *et al*, 1953, Weston *et al*, 1953, Wrong, 1956)

The second possibility is that an abnormal hypotonicity of the cells determines the hypotonicity of the ECF (Sims *et al*, 1950, Rapoport, West and Brodsky, 1951)

McCrory and Macaulay (1957) described an infant with diffuse cerebral damage and hyponatraemia Her ECF

of the low plasma [Na], on 19th June he excreted 210 m-equiv. of

Muscle analysis

The question whether the total sodium content of the body was low, or normal, but diluted by an excess of water in the ECF could be settled by an analysis of muscle.

Table I

ANALYSES OF MUSCLE FROM THE TWO PATIENTS, COMPARED WITH "NORMAL" VALUES

	Water per cent	m-equiv /kg fat free tissue Cl	Na	K
Albert	74 4	31 8	45 5	91 2
Ivor	79 1	27 2	47 9	89 3
Talso, Spafford and Blaw (1953)	77 6±0 6	19 1±3 9	33 7±6 4	94 0±5 9
Wilson (1955)	77 5	25 6±5 1	40 0±6 0	92 3±7 6
Barnes Gordon and Cope (1957)	80 3±1 6	23 1±6 5	43 6±11	91 3±8 3

ANALYSIS OF PLASMA TAKEN FROM THE TWO PATIENTS AT THE TIME OF THE MUSCLE BIOPSY

	m equiv /l. Cl	Na	K
Albert	89 6	124	5 5
Ivor	87 6	124	3 7

The specimens were taken from paralysed muscles in both patients The electrolyte contents are shown in Table I, with "normal" values for specimens taken from anaesthetized

blood pressure was 180/80. His fits were rapidly controlled, but he then had a bilateral spastic paralysis with extensor plantar responses, and never regained consciousness On the second day he stopped breathing and respiration had to be maintained with a Beaver respirator for 12 hours. Subsequently he had a purulent bronchopneumonia and on the fourth day a tracheotomy was done to enable a clear airway to be maintained by suction The bladder was kept drained by a Foley catheter but the urine was not infected until the last days of his illness He died on 11th August of bronchopneumonia

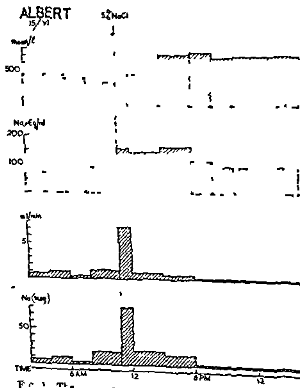
At post mortem there was a large area of softening in the left temporal lobe The vessels of the circle of Willis were very atheromatous There was evidence of an earlier hypertension, the left ventricle was hypertrophied to a thickness of 22 mm compared to 8 mm in the right ventricle, and the kidneys showed hypertensive changes There was remarkably little evidence of infection in them although there was a purulent cystitis

Albert was certainly water deficient in the early days of his illness

blood was 55 per cent He was then given six litres of water in two days, the packed cell volume fell to 41 per cent and the plasma [Na] fell to 128 m equiv /l Subsequently his plasma [Na] fluctuated between 130 and 110 m equiv /l The blood urea was 34 mg per 100 ml and the creatinine clearance 70 ml /min

Ivor, aged 54, was admitted on 11th June 1957 having been ill for 18 days with acute peripheral neuropathy affecting mainly the motor nerves and accompanied by an enlargement of the liver The plasma albumin (2nd July) was 2 9, and the total protein 6 g per 100 ml The cause of his illness was not discovered In the next five days he developed a partial respiratory paralysis with bronchopneumonia His blood pressure, which had been normal, fell to 90/60 Subsequently he was fed by tube, and his purulent bronchial secretion was aspirated through a tracheostomy At the end of June he began slowly to recover and was taking some food by mouth on 4th July, but almost immediately had a *severe relapse* Tube feeding continued until the end of July, by which time he was able to move his limbs, though they were sti

tonic saline (Crawford and Ludemann, 1951; Birchard, Rosenbaum and Strauss, 1953, Papper *et al*, 1956), and depends, of course, on the liberation of ADH (Holland and Stead, 1951)



F... Th...

Water deprivation

When fluid was withheld for 19 hours both patients produced a urine of small volume and high osmolality (Tabl... Th...

patients The potassium content was normal The sodium content, far from being lower than normal was in fact at the upper limits of the normal The chloride content was similarly high For this to happen with a low concentration of sodium in ECF, the amount of ECF in the muscle samples must have been larger than normal

Hypertonic saline

The infusion of hypertonic saline produced only a transient increase in the plasma [Na]

The response was studied in detail in *Albert* He had *500 ml* of 5 per cent sodium chloride (436 m equiv) infused over about three hours on 15th June when his plasma [Na] was initially 127 m equiv /l (Fig 1)

The immediate response to this infusion was an osmotic diuresis with an output of 7 3 ml /min of urine containing 330 m osm and 155 m equiv of sodium per litre During the infusion he excreted 80 m equiv of sodium The plasma [Na] increased to 143 m equiv /l during the infusion and was 138 m equiv /l at the end In the following 21 hours he responded quite differently He excreted only 55 m equiv of sodium and his urine flow fell to 0 2 ml /min with a concentration of 696 m osm /l He was thus retaining water and diluting the sodium he had retained Three days later his plasma [Na] was again only 130 m equiv /l

Ivor had infusions of *300 ml* of 5 per cent sodium chloride *on 22nd June and 510 ml on 24th June We did not make* very detailed studies of his response but the plasma [Na] before and after the second infusion was 113 and 115 m equiv /l During the first three hours of this infusion when he had received 190 m equiv he excreted only 80 m equiv Both the infusions were followed by a retention of water

These are not the responses one would expect from salt depleted patients (Black Platt and Stanbury, 1950) They imply that the osmolality of the body water was being maintained even at the expense of increasing the volume of the extracellular fluid This is the normal response to hyper

tonic saline (Crawford and Ludemann, 1951, Birchard, Rosenbaum and Strauss, 1953, Papper *et al*, 1956), and depends, of course, on the liberation of ADH (Holland and Stead, 1951)

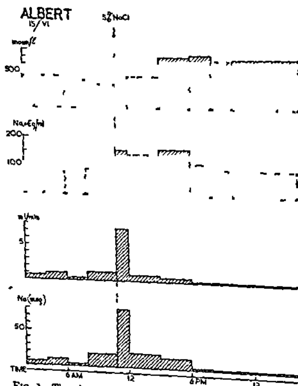


FIG 1 The changes in total sodium excretion urine flow sodium concentration and osmotic concentration of the urine after the infusion of 500 ml of 5 per cent sodium chloride (436 m-equiv)

Water deprivation

When fluid was withheld for 19 hours both patients produced a urine of small volume and high osmolal...
The ...

low The ratio of urine to plasma osmolalities, which can normally rise to about 4 with water deprivation, was 3 7 in Albert and 3 3 in Ivor The deprivation of water was associ-

Table II

EFFECTS OF DEPRIVING THE TWO PATIENTS OF WATER FOR 10 HOURS

Changes in urine and plasma	*Albert*	*Ivor*
Maximum urine concentration (m osm/l)	904	870
Flow at maximum concentration (ml /min)	0 27	0 11
[Na] (μ equiv /ml of urine)	22 6	14 6
Plasma (m osm /l)	243	267
Change in plasma ([Na] m equiv /l)	112–117	112–123

ated with a great reduction in the renal excretion of sodium, and the increases in the plasma [Na] were unexpectedly large They were not maintained however, for the plasma *[Na] had returned to the original levels after 48 hours*

Effect of water

The effect of a water load was adequately tested only in Albert, who on 15th July received one litre of water in 30 minutes, by stomach tube He excreted all of this water in less than three hours, achieving a diuresis of 7 3 ml /min, with an osmolal concentration of 54 m osm /l , and a sodium concentration of 4 m equiv /l These low concentrations are similar to the minimum values obtained in normal persons (Schoen, 1957) The values for the plasma sodium before and after the test were 115 and 112 m equiv /l Remarkably low osmolal concentrations were found twice in the 24 hour collections of urine from Albert The values, 153 and 168 m osm /l , were lower than in the plasma, in spite of the fact that at these times the plasma [Na] was exceptionally low, *104 m equiv /l , these values were obtained on the days* immediately following administration of pitressin (see below)

We did not find any very low urinary concentrations in eight 24 hour collections from Ivor that were tested In one specimen an osmolal concentration of 215 m osm /l was the same as that of the plasma taken at that time

Effect of potassium chloride

In view of Laragh's (1954) findings of a rise in plasma [Na] with the administration of potassium chloride in patients with hyponatraemia we gave 100 m equiv of potassium chloride on two successive days to both the patients There was no increase in the plasma [Na] and only a slight rise in the plasma [K]

The data so far reported show that the renal excretion of sodium could be made to vary from very small to very large amounts and in particular although sodium continued to be excreted while the plasma concentration was low, the kidneys were able to conserve sodium during the dehydration reaction But a rise in the plasma [Na] produced by hypertonic saline was followed by retention of water which restored the osmolality of the plasma to its original level

Effect of pitressin

All these results might be taken to show that these patients had an intact antidiuretic mechanism which operated to maintain their plasma osmolality at a lower level than normal Their response however to exogenous ADH given as pitressin was quite unexpected

After one litre of water by intragastric drip the patients received 100 m u of pitressin intravenously and 5 i u of pitressin in oil intramuscularly Urine was collected in hourly periods for the following five hours the gastric drip was running throughout but the amounts given after the initial load were unfortunately not recorded The results are shown in Table III In Fig 2 they are compared with the results of water deprivation Ivor began with a concentrated urine, but after the first hour the maximum osmolality achieved after pitressin was some 500 or 600 m osm. less than after dehydration The effect of pitressin was tested a second time in Albert and he then passed urine with a concentration of 215 m osm /l that is lower even than his own hypotonic plasma The low concentration of urine in these tests depended on the comparatively high urine flow, and not on a

reduced excretion of solutes The rate of excretion of sodium and of solutes was actually higher than with dehydration, though lower than immediately before the pitressin was

Table III

EFFECTS OF PITRESSIN IN THE TWO PATIENTS WHILE THEIR HYDRATION WAS MAINTAINED

Changes in urine and plasma	*Albert*	*Ivor*
Maximum urine concentration (m osm /l)	280	387*
Flow at maximum concentration (ml /min)	1 5	2 4
[Na] (μ equiv /ml of urine)	33 2	60 2
Plasma (m osm /l)	233	243

* The results on the first collection (see Fig 2) have been neglected

given Glomerular filtration rates were not measured The *same batch of pitressin was shown to have normal activity in other subjects*

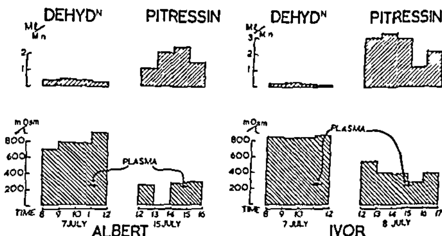


Fig 2 Comparison of the changes in the flow and concentration of urine following deprivation of water and following pitressin and a water load

The difference between the effects of water deprivation and pitressin is far greater than anything observed in normal people (Jones and de Wardener, 1956), and indeed indicates an almost complete failure to respond to pitressin in the

presence of a water load while the response to water deprivation was nearly normal Pitressin was not entirely without effect on the urine flow since this diminished

The failure of Albert and Ivor to respond to pitressin might represent the human counterpart of the experiments of Wesson and co workers (1950) Their dogs with an isotonic expansion of the ECF did not respond to pitressin

We have mentioned that the failure of response was not a complete one and it therefore remains possible that the original expansion of the ECF represented an effect of the patients own ADH as Schwartz and co workers (1957) postulated for their two cases Although Schwartz and co workers do not remark on it there were occasions when their patient W A like Albert produced a hypotonic urine following an additional expansion of the ECF These observations would be consistent with the suggestion that when the ECF is expanded beyond a certain point the kidneys become refractory to the action of ADH

If we assume that an overproduction of ADH was reponsible for the hypotonicity of the ECF in Albert and Ivor the alternatives previously suggested still remain whether the stimulus to ADH production represented a homeostatic mechanism for maintaining a hypotonic ECF in two people who might have had hypotonic cells or whether it represented a response to an abnormal stimulation of some unidentified receptor

Summary

The problem of hypernatraemia seems in general to be one of water deficiency That of hyponatraemia is sometimes one of salt deficiency but often one of excessive dilution of the ECF with water The latter seems to have been the fault in the two patients we studied Muscle biopsies revealed normal or high sodium contents In their responses to hypertonic saline water deprivation and water loading their homeostatic mechanisms were adjusted to maintain an abnormally large volume of ECF with low tonicity Though

reduced excretion of solutes The rate of excretion of sodium and of solutes was actually higher than with dehydration though lower than immediately before the pitressin was

Table III

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Maximum urine concentration (m osm /l)	280	387*
Flow at maximum concentration (ml /min)	1 5	2 4
[Na] (µ equiv /ml of urine)	13 2	60 2
Plasma (m osm /l)	283	293

* The results on the first collection (see Fig 2) have been neglected

given Glomerular filtration rates were not measured The same batch of pitressin was shown to have normal activity in other subjects

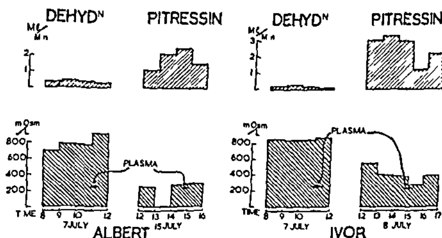


Fig 2 Comparison of the changes in the flow and concentration of urine following deprivation of water and following pitressin and a water load

The difference between the effects of water deprivation and pitressin is far greater than anything observed in normal people (Jones and de Wardener, 1956), and indeed indicates an almost complete failure to respond to pitressin in the

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they produced a hypertonic urine of low volume when deprived of water, they did not always produce a hypertonic urine with pitressin and water. Under certain circumstances, therefore, the kidney can excrete a hypotonic urine in the presence of pitressin while retaining its ability to respond normally to dehydration

Acknowledgements

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DISCUSSION

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take up any sodium that is in the gut [illegible] stools from [illegible] with the salt losing type of adreno- [illegible] examined [illegible] to be much

...natraemia. The urine
One finds both
hat might seem

intestine?

Desaulles I have no precise data.

Black I want to express agreement with Dr Fourman, because I think that none of the alleged clinical tests for water depletion, such as the
... another possible cause of

Fourman I think that is a very interesting comment The very severe dehydrations probably do produce renal lesions and we have been wondering whether that accounts for the systemic acidosis, which is so often a prominent feature

Wallace Chloride acidosis always occurs

Fourman What is the plasma bicarbonate?

Wallace In our experience it is always low Chloride is making bicarbonate forfeit its place in serum

Desaulles Dr Fourman, was it possible to make steroid determinations in your case?

We have made an observation on animals that is not identical but may point in the same direction as the observation you have made If [illegible]

the sensitivity to pitressin [illegible]

urine was normal A Gowenlock in Manchester measured the aldosterone output in one of our patients and it was normal We also did 17-ketosteroid assays as a crude measure of their corticoid output, and the results were normal It is obvious that the hyponatraemia does not lead to a stimulation of the aldosterone output of the adrenal

Desaulles Could this be given the same interpretation as the findings of Prader, Spahr and Neher (1955 *Schweiz med Wschr*, 85, 1085)? There may be some form of sodium losing syndrome

Adolph It seems to me Dr Fourman that in order to show that there is something more to one of these syndromes than a lack of drinking behaviour or drinking response on the part of the individual, you have to perform your tests in a certain order you have to be sure that the patient has plenty of water when you do the salt test and plenty of salt when you do the water test Could you have switched the tests around and still obtained the same results?

Fourman The saline load was done three weeks before the dehydration The dehydration preceded the pitressin by one day in one of the patients, by a week in the other patient The pitressin test was accompanied by a load of water at the time I do agree that one test can influence another but I do not think that they did in this instance

Borst When a high or a low sodium concentration in the blood plasma is maintained we believe that this is almost always due to an insufficient circulation This insufficiency often results from dehydration but it may have other causes such as cardiac failure or hypoproteinaemia We found a high blood sodium in anaemic patients who had had recurrent haemorrhages from peptic ulcer They had no free access to water and had been treated with abundant saline infusions they had substantial oedema During several days the urine contained less sodium than tap water After a large transfusion of blood the sodium excretion started and the blood sodium fell to a normal level Simultaneously, the output of water increased and the urea concentration of the urine which had been very high decreased The counterpart was

A large blood transfusion elicited a considerable water diuresis and the blood sodium rose to normal, while the oedema fluid was excreted.

With both the high and the low sodium concentrations the circulation was inadequate. In the first instance the excess of sodium and a less considerable excess of water was excreted as soon as the normal blood volume was restored In the second the rise in blood volume led to the [illegible]

salt and water has an effect on the circulation The problem is that an excess or an inadequacy of the circulation in patients cannot be measured in a satisfactory way Since this factor cannot be disregarded we have to estimate it on the basis of indirect evidence

GLANDULAR SECRETION OF ELECTROLYTES

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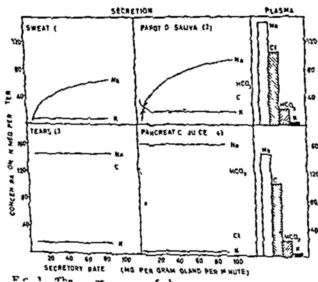
THE ducts or tubules of glands with external secretion are usually quite complex in structure and morphologically they differ to a considerable extent from gland to gland It is, therefore, reasonable to assume that the ducts do not merely serve as pathways for the secretion formed in the acini, but that they contribute somehow to the elaboration of the final secretory product This possibility has already been considered in the past century by Merkel (1883), mainly on morphological grounds, and by Werther (1886), who made a comparative investigation of the concentration of salt in various types of saliva The results of these experiments were however inconclusive, and in 1950 Babkin restated the need for a study of the physiology of the glandular ducts Since then certain advances have been made through comparative work, by the application of concepts from modern renal physiology and with the use of electrophysiological methods, relating changes in membrane potentials to ionic transport It is the purpose of the present paper to review this work and to present a theory of the mechanism of glandular electrolyte secretion based on the available data

Fig 1 shows a comparison between the concentrations of the main electrolytes in sweat parotid saliva, tears and pancreatic juice in relation to secretory rate calculated in milligrams per gram gland per minute The following similarities and differences between the four secretory products are apparent from Fig 1

The Excretion of Sodium

In sweat and in parotid saliva the concentration of sodium is smaller than the concentration of sodium in plasma and

varies with the rate of secretion With increasing secretory rate the concentration of sodium rises to about 60 m equiv /l in the sweat and to about 90 m equiv /l in the parotid saliva, but no definite maximum is reached in either secretion This finding conforms with the old work of Heidenhain (1868) Langley and Fletcher (1889), Kittsteiner (1911, 1913), and Hancock Whitehouse and Haldane (1929)



F . 1 The f

In tears and in pancreatic juice the concentration of sodium in secretion water is about equal to the concentration of sodium in plasma water and is independent of the rate of secretion

The Excretion of Potassium

The concentration of potassium in all four secretions is independent of wide ranges of variation in secretory rate

In parotid saliva however, a definite rise in potassium concentration is noted at rates smaller than 15 mg per gram gland per minute. This finding is in agreement with the results of Langstroth, McRae and Stavraky (1938) and Burgen (1956). A similar rise in potassium concentration possibly occurs at low rates of sweat secretion (Kuno 1956) but could not be demonstrated with the experimental technique employed by Schwartz and Thaysen (1956). In the two other secretions a rise in potassium concentration at low secretory rates has never been observed.

The Excretion of Anions

The main anion of sweat and tears is chloride. This anion accounts for about 80 per cent of the sum of the concentrations of sodium and potassium in the tear fluid. Chloride concentration of sweat is not depicted in Fig. 1, but Locke and his co-workers (1951) found the following relation: sodium = *1.12 chloride + 3 m equiv./l.*

The chief anion of parotid saliva and pancreatic juice is bicarbonate. With increasing secretory rate the concentration of *bicarbonate rises in both secretions and reaches* a maximum of about 60 m equiv./l in parotid saliva and about 90–130 m equiv./l in pancreatic juice. When this maximum concentration (which is subject to individual variation) has been arrived at the concentration of bicarbonate remains independent of further increases in the rate of secretion. The concentration of chloride varies inversely with that of bicarbonate. In both secretions and at all rates the sums of the concentrations of the two anions equal about 80–90 per cent of the sums of the concentrations of sodium and potassium.

The following hypothesis has been put forward to explain the demonstrated differences in the excretion of the main cations. In all four glands a precursor solution is formed in *which the concentration of sodium is independent of the rate* of precursor formation. In the sweat and parotid glands but not in the other two glands sodium is consequently reabsorbed *by a process of a limited maximal capacity* (Thaysen

Thorn and Schwartz, 1954, Thaysen, 1955, Schwartz and Thaysen, 1956, Bulmer and Forwell, 1956, Bro Rasmussen, Killmann and Thaysen, 1956) Like sodium, potassium is transferred into the precursor at a constant concentration, but it is not reabsorbed in any of the glands The rise in potassium concentration at the low secretory rates in parotid saliva (and in sweat?) may be secondary to reabsorption of water from the precursor as indicated by Langstroth, McRae and Stavraky (1938) and by Thaysen, Thorn and Schwartz (1954) and/or to an exchange between sodium and potassium ions during the process of sodium reabsorption

Table I

COMPARISON BETWEEN THE CALCULATED CONCENTRATIONS OF SODIUM AND POTASSIUM IN THE PRECURSOR SECRETIONS OF FOUR SECRETORY PRODUCTS AND THE CONCENTRATIONS OF THE SAME IONS IN PLASMA WATER

	SWEAT	PAROTID	LACRYMAL	PANCREATIC	PLASMA WATER
Na	79	112	146	161	160
K	9	19	15	5	5
SUM	88	131	161	166	165

Fig 2 shows a linear regression of the rate of sodium excretion in parotid saliva on the rate of secretion According to the above hypothesis the values for slope and intercept in Fig 2 can be interpreted to mean that sodium is transferred into the precursor solution at the rate of 0 112 microequivalents per mg of saliva discharged and that 2 4 microequivalents are subsequently reabsorbed per gram gland per minute The sodium *concentration* of the sweat precursor has been calculated in a similar manner from the data of Schwartz and Thaysen (1956) and the values are compared to those of the other secretions and to plasma water in Table I According to Table I the sums of the concentrations of sodium and potassium in the presecretions of saliva and sweat are lower than the sums of the concentrations of the same ions in the two other secretions and in plasma water No other cations

are present in parotid saliva and in sweat in sufficiently large concentration to make up for this difference Judging from the results of Table I, the production of sweat and parotid saliva should therefore involve secretion of hypotonic precursor solutions, a process which *a priori* does not appear very likely

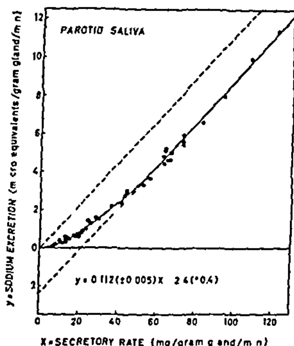


FIG 2 The relation between the rate of sodium excretion in parotid saliva (in microequivalents [illegible]

glan l j er minute

It must be emphasized, however, that the calculated figures for precursor sodium concentration (and sodium reabsorption) in the sweat and parotid glands underestimate actual values, since the regressions for sodium excretion on secretory rate have been fitted to points which approach, but do not reach, a rectilinear relationship within the observed range (cf Fig 2) One explanation for this considerable splay in

the observed values from the asymptote could be that there is a certain back diffusion of water in the sequence of active sodium reabsorption As demonstrated below there is reasonable qualitative evidence to suggest that water is, in fact, reabsorbed from the precursors of sweat and parotid saliva

Fig 3 illustrates that the concentration of urea in sweat, tears, and parotid saliva remains proportional to the con-

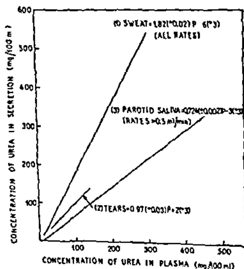


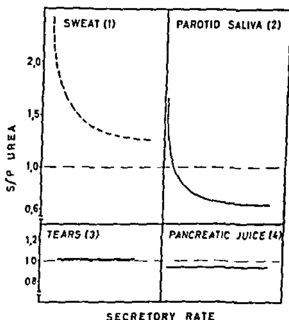
FIG 3 The relation between the concentrat...

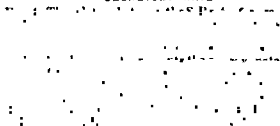
centration of urea in the plasma within a wide range of variation in the latter This finding indicates that urea is excreted in these secretions by a process of simple diffusion and not via a specific secretory mech...

by increasing load ...
as a tracer for the ...
glands in a similar ... as in the glomerular nephron

Fig. 4 shows the relationship between the S/P (secretion/plasma) concentration ratio for urea and the rate of secretion of sweat, parotid saliva, tears and pancreatic juice.

In tears and in pancreatic juice there is apparently diffusion equilibrium between the secretion and the plasma at all





rates of glandular activity. On the basis of these findings no statement can be made about the existence or non-existence of an internal circulation of water in these glands.

In sweat and in parotid saliva S/P urea varies with the rate of secretion. In the sweat S/P urea decreases from 2 or 3 at the low secretory rates to about 1 when sweating

becomes profuse (Araki and Ando, 1953, Bulmer, 1957) In parotid saliva the ratio decreases from about 1 6 at low rates of secretion to about 0 6 when the flow of saliva is brisk (Albrectsen and Thaysen, 1955) Since no specific secretory mechanism for urea exists in either gland, it is reasonable to conclude that urea which is diffusing into the gland with some precursor solution, is raised to a concentration greater than that of the plasma by reabsorption of water from the precursor in a region of the gland which is less permeable to urea than the site of precursor formation The rate of change in S/P urea with secretory rate suggests that water reabsorption represents a relatively constant quantity at all rates of precursor formation and it is not unreasonable to assume that the reabsorption of water occurs as a mere passive sequence of active sodium reabsorption

Quantitative information about precursor formation and water reabsorption can however, hardly be gained from these results or from similar "clearance" studies with other solutes Morphological and physiological evidence strongly argues against the possibility that the secretion precursor represents an ultrafiltrate of the plasma like the urine precursor of the glomerular nephron A "glandular inulin" probably does not exist and it is quite possible that exact knowledge about the composition of the precursor secretions and about the manner in which they are modified as they flow down the glandular ducts can only be obtained by micropuncture techniques

However Lundberg (1955 1957*a b c*) work ng on th

... that sodium is reabsorbed from a precursor secretion in some of the duct possessing glands

In the submaxillary gland, which produces a secretion in which sodium concentration varies with secretory rate in about the same manner as in parot d sal

gland is activated by stimulation of the chorda. A similar internal duct negativity could not be demonstrated in the sublingual gland (Lundberg, 1957a), which (like the lachrymal and pancreatic glands) produces a secretion that is isotonic with the plasma and has a sodium concentration of about 150 m equiv./l. Provided that the potential changes on stimulation can be regarded as the electrical signal of ionic transport, Lundberg (1957a) concludes that there is a net transport of cation from the lumen to the blood side in the ducts of the submaxillary gland, but not in the sublingual gland. Although the composition of the submaxillary secretion was not measured simultaneously with the duct potential, the latter appears large enough for it be to accepted that the reabsorption of anion is merely a passive sequence of active cation transport.

With one microelectrode inserted into acinous cells and the other electrode on the gland surface, Lundberg (1955, 1957a) detected a considerable increase in the negativity of the acinous cells on stimulation of the submaxillary as well as of the sublingual gland. The lumen of the acini, likewise, becomes negative as compared to the morphological interior, but this negativity decreases slightly with continued stimulation of the gland. These potential changes may be due to a net transport of anion from the blood side into the glandular lumen. In another paper Lundberg (1957c) directly demonstrated this anionic dependence of secretion and secretory potentials in the perfused sublingual gland. Substitution of sodium chloride with sodium nitrate or sodium thiocyanate caused the secretion to stop almost entirely and decreased the potential changes. The secretory response and the potentials reverted to normal when sodium chloride was again added to the perfusate.

On the basis of the experiments quoted in the present report, it appears reasonable to suggest the following mechanism for the secretion of electrolytes and water by the duct possessing glands. Active outward transport of anions is a main factor in the formation of the secretory products of all

glands In some glands the chief anion transported is chloride (sweat, tears sublingual saliva), in others bicarbonate ions are added in varying proportion, possibly due to the presence of carbonic anhydrase in the cells (pancreatic juice, parotid saliva submaxillary saliva) It is reasonable to assume that water moves in a merely passive sequence of ionic transport from the blood side into the glandular lumen, and that the presecretions of all glands are isotonic or nearly isotonic

In certain glands (sweat, parotid and submaxillary) sodium is reabsorbed from the precursor secretion as it flows down the glandular duct system and it is likely that anions move from duct lumen to the blood side in a passive sequence of the active sodium reabsorption The chief anion reabsorbed in this manner appears to be chloride, independently of whether the primary secretion contains primarily chloride or primarily bicarbonate ions It can be seen from a glance at Fig 1 that the parotid and the pancreatic glands apparently form pre secretions of qualitatively similar composition, and that the main difference in the anionic pattern of the final secretory products is that chloride ions have been removed from the saliva precursor As a consequence of active sodium re absorption a certain quantity of water is, moreover, diffusing back into the blood stream, although it is obvious that water reabsorption does not occur isotonically as in the [illegible]

[illegible] transports in the duct possessing glands According to Fig 5 it is however, not unreasonable to suggest that sodium reabsorption is located in the striated intralobular ducts Striated epithelium is present in the parotid and submaxillary glands which apparently reabsorb sodium but it is absent in the sublingual, pancreatic and lachrymal glands which show no evidence of sodium re absorption The precursor secretions are probably formed by the acini as well as by the cuboidal epithelium of the intercalary ducts, the former producing a viscous secretion with a high concentration of organic material, the latter a

watery secretion with a low concentration of organic material (cf. Babkin, 1950). With respect to the sweat gland it is

SWEAT	PAROTIS	SUBMAX.	SUBLING	PANCREAS	LACRYMAL
$S_{Na} < EC_{Na}$			$S_{Na} = EC_{Na}$		
S_{Na} varies with secretory rate			S_{Na} independent of secretory rate		

suggested that precursor formation is located in the coil, whereas reabsorption of sodium takes place in the duct.

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Physiol., 38, 293

DISCUSSION

Dawson: As far as I am

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rates of secretion Certainly this is not the case in the parotid gland (Fig 4, p 68) Conversely, I do not venture to claim that the cells in the duct are impermeable to an extent that would completely prevent urea *from diffusing back into the blood stream along the concentration* gradient created by water reabsorption But the amount of urea diffu-

slowly over others This difference is not important when one measures total body water as the volume of distribution of urea, because one waits until complete equilibrium has been established before the measurement is made But the difference is important in the rate-dependent process of secretion, where the time available for diffusion becomes limiting

Karvonen In prolonged sweating the potassium concentration is higher to start with and then gradually decreases There is no similar change in sodium or chloride and that would agree quite well with the reabsorption and consequent storing of potassium in the tubule, whereas sodium and chloride are not stored (Ahlman *et al* (1953) *Acta endocr., Copenhagen*, 12, 140)

Thaysen Yes, the first sample of sweat obtained after stimulation may have a higher potassium concentration than the following ones One reason for this may be that the first sample is contaminated with cellular debris, sebum and sweat residues on the skin surface

speculated that the vigorous flow of saliva, caused by stimulation, "pushed out" first a small amount of secretion, which had been produced

sodium concentration falls The net amount of sodium lost from the

Physiol, 8, 621)

Adolph Can somebody clarify the reports that tears are very *hypertonic* when they are formed?

Dawson I did some analyses a long time ago, and we discovered that the chloride concentration was equal to that of the blood. It is a very difficult problem obtaining tears, because you have got to make the person cry very hard to get enough to do an analysis

and not just filtered from the plasma. One then wonders whether epithelium does not similarly push out potassium in exchange for the sodium which is being reabsorbed—the sort of mechanism that is possibly under aldosterone control. [illegible]

Thaysen: An exchange mechanism between sodium and potassium ions at the site of sodium reabsorption is certainly a very likely possibility. [illegible]

[illegible] change for reabsorbed sodium ions. Similarly, the adrenal steroids may have a dual site of action in the glands. In contradistinction to the situa- [illegible] There is some [illegible]

As regards your comment about the rice diet, sodium depletion, induced by a low sodium diet, causes the concentration of sodium to [illegible] increase in sweat as well [illegible]

and incomplete as compared to that of the kidney tubule (Robinson *et al.* (1955). *J. appl. Physiol.*, 8, 159, Thorn *et al.* (1956) *J. appl. Physiol.*, 9, 177).

Karvonen: Can anyone comment on the statistical finding that men [illegible] ten in their sweat, and that [illegible] er than in men (Ahlman *et* [illegible] allenge. We have similar findings in animals, not in sweat but in urine, but I have absolutely no explanation for it. It is just an observed fact.

Talbot: I wonder if those who are commenting on the sodium, chloride and potassium concentrations in sweat all have in mind the relationship

between rate of sweating and the concentration, because it varies enormously.

Thaysen: Yes, that is very important. Secretory rate must be controlled in all work on electrolyte composition of secretions, and compara-

[illegible]

[illegible] of other factors. What I mean [illegible]

comparative work it is a prerec[illegible]

rate, but also the number of fu[illegible]

[illegible] *Acta*, 95, 129)

and not just filtered from the plasma. One then wonders whether epith- [illegible]

Thaysen: An exchange mechanism between sodium and potassium ions at the site of sodium reabsorption is certainly a very likely possibility. This may be one factor causing the potassium concentration of the final secretory product to exceed that of the plasma. However, glands which [illegible]

[illegible] of the plasma [illegible] some [illegible] in ex- [illegible] ls may [illegible] situa- [illegible] gland [illegible]

[illegible] There is some [illegible]

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and incomplete as compared to that of the kidney tubule (Robinson *et al.* (1955). *J. appl. Physiol.*, 8, 159, Thorn *et al* (1956). *J. appl. Physiol.*, 9, 477).

[illegible] men [illegible] at [illegible] *et* [illegible] ar [illegible] no explanation for it. It is just an observed fact.

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and salt There does not appear to be any inability to secrete ADH or adrenocortical hormones, though it is possible that the infant does lack the power to adjust the amounts secreted with any precision The endocrinological situation, therefore, is essentially one of target organ insensitivity due to immaturity

An interesting hypothesis relating to neonatal weight loss has been put forward by Gans and Thompson (1957) These workers measured the urine output and its content of oestrogens and 17 hydroxycorticosteroids in six normal male neonates during the first few days of life The findings were similar in all the infants Large amounts of oestriol (up to a milligram or more) were excreted on the first post partum day, the quantity falling rapidly during the next two or three days to the order of 1 or 2 μg by the sixth day Oestrone and oestradiol were found to the extent of 1–2 μg during the first and second days and then disappeared There was a decreasing excretion of urine during the first three to five days, and by the end of this time, postnatal weight loss had ceased The excretion of 17 hydroxycorticosteroids showed only minor fluctuations throughout The specific gravity of the urine was low at first but became more concentrated as the excess of water was excreted in spite of the fact that fluid intake was increasing during this time

Gans and Thompson suggest that part at least of the hydraemia of the newborn infant is due to water retention caused by the high circulating oestrogen level—the oestrogens being, of course, of maternal origin As the oestrogens are excreted the fluid excess is eliminated

Adrenal hyperplasia

Adrenal hyperplasia is a disorder with a definite predilection for the female sex In Wilkins' series (W [illegible]

[illegible] about one [illegible] of these patients have a tendency to loss of sodium and

HORMONAL ASPECTS OF WATER AND ELECTROLYTE METABOLISM IN RELATION TO AGE AND SEX

G I M SWYER

Obstetric Hospital University College Hospital London

NEARLY all the hormones may have some influence on water and electrolyte metabolism However, for most of them this effect is indirect and occurs only under highly abnormal circumstances Thus, the dehydration which exists in uncontrolled diabetes mellitus or in hyperparathyroidism is the result, respectively, of gross deficiency of insulin or excess of parathormone, and certainly does not point to any physiological rôle of these hormones in water metabolism The same is essentially true of thyroid hormone and though perhaps with reservations, of the sex hormones and gonadotrophins Only posterior pituitary antidiuretic hormone (ADH) and *certain of the adrenocortical steroids are directly concerned* with the day to day and minute to minute adjustments needed to maintain fluid and electrolyte homeostasis in mammals The major details of this hormonal control are well known and it is not necessary to relate them here It is proposed, on the other hand, to examine how the influence of hormones on fluid and electrolyte balance differs at various ages and in the two sexes In general it is fair to say that little attention has been paid to considerations such as these, and for the most part knowledge is meagre

In Infancy

Fluid and electrolyte control is notoriously inefficient at birth and during the first few weeks or so of life The late development of the loop of Henle is generally considered to be responsible for this (Hubble, 1957) the infant kidney being unable, in consequence, to vary tubular reabsorption of water

and salt There does not appear to be any inability to secrete ADH or adrenocortical hormones, though it is possible that the infant does lack the power to adjust the amounts secreted with any precision The endocrinological situation, therefore, is essentially one of target organ insensitivity due to immaturity

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Adrenal hyperplasia

Adrenal hyperplasia is a disorder with a definite predilection for the female sex In Wilkins' series (Wilkins 195[illegible]

[illegible] about one [illegible]ourth of these patients have a tendency to loss of sodium and

to elevation of the plasma potassium, as a result of which early death may occur from dehydration and circulatory collapse, *or from cardiac arrest due to hyperkalaemia* Once again, the number of females affected is some three times that of males

The mechanism for this sodium loss is not understood Very likely there is a defect in aldosterone synthesis, but it is also possible that some of the abnormal steroids produced by the hyperplastic adrenals may actually cause sodium loss It is well known that surprisingly large amounts of sodium chloride and cortexone acetate (DOCA) may be needed to remedy the electrolyte defects in these infants, suggesting that more than mere replacement of deficient hormone is necessary However, the response to 9α fluorohydrocortisone, together with cortisone, may be far more satisfactory In a 1½ year old patient of the writer's, a female pseudohermaphrodite with the salt losing disorder, 10 mg daily of DOCA intramuscularly, together with large sodium supplements, was necessary to maintain electrolyte balance With only 0 25 mg of 9α fluorohydrocortisone daily by mouth, it was possible to maintain balance with no sodium supplement at all

A small proportion of patients with adrenal hyperplasia (about 6 per cent) may show hypertension It is possible that in these there is actually sodium retention Bongiovanni and Eberlein (1955) *have demonstrated in such a patient* a defect in the synthesis of cortisol different from that usually found in adrenal hyperplasia This patient was producing increased amounts of cortexone and 17 hydroxycortexone, it is thought probable that these steroids were responsible for the hypertension

Changes in Relation to Adolescence

Knowledge of endocrine changes in relation to adolescence is rather sketchy It is ably summarized by Tanner (1955) The impact of these changes on fluid and electrolyte metabolism is somewhat obscure Certain morphological changes of

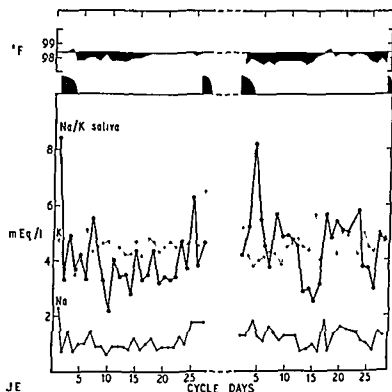
possible significance occur Thus, a considerable growth spurt in the weight of the adrenal gland, more in boys than in girls, has been observed It is almost entirely due to growth of the cortex The weight of the thyroid also shows an adoles cent spurt, but without any sex difference Scanty data on hormone excretion indicate a slow increase in the excretion of oestrogen in both boys and girls during childhood, with a marked increase at puberty in the case of girls, while in boys the rate of increase hitherto manifested is merely maintained Androgen excretion is similar in the two sexes before puberty, after puberty there is a marked rise in the case of boys, but a not unimportant rise also occurs in girls, no doubt as a result of increased adrenocortical activity There is a gradual rise in the rate of secretion of adrenal corticoids, without sex dif ference, from birth to maturity The increase appears to be proportionate to body size, without any adolescent spurt The blood level of 17 hydroxycorticosteroids is much the same at all ages and the responsiveness of the adrenals to stimulation by adrenocorticotrophic hormone is also un affected by age, except, of course, in so far as the adrenal glands are smaller in children than in adults A steady fall in the serum protein bound iodine over the years six to 15 parallels the fall in basal metabolic rate, and the precise [illegible]

Effects of the Menstrual Cycle

An important sex difference is introduced by the cyclic variations in hypothalamic pituitary-ovarian (and perhaps adrenocortical) function which determine the menstrual cycle in females It might well be expected that these would lead to important fluctuations in fluid and electrolyte balance

Variations in body fluid during the menstrual [illegible] been [illegible]
of

reported weight gains of up to 14 lbs. at or during menstruation in two women. Several other writers (see Chesley and Hellman, 1957) have concluded that approximately 30 per cent of women have weight gains associated with menstruation. The suggestion that premenstrual weight gain is due to



water and salt retention, mediated by oestrogens, is due to Thorn, Nelson and Thorn (1938). Long and Zuckerman (1937) postulated a rôle of adrenal salt-retaining hormones in the electrolyte imbalance causing premenstrual fluid retention.

In a recent investigation, Chesley and Hellman (1957)

studied 23 normal young women and found that in one third of them the weight was maximal during the premenstrual eight days—in accordance with earlier writers Closer analysis, however, failed to substantiate the physiological basis of such weight gains, since, when they did occur, they

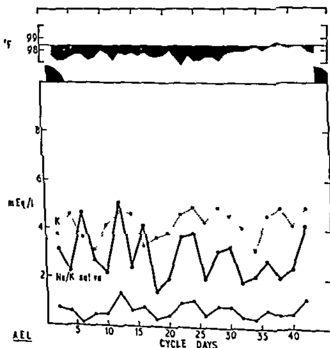


FIG 2. A long but ovular, cycle in a normal woman

were slight and were not repeated from one cycle to the next It was further shown that the incidence of premenstrual weight gain was the same as would be ...

any cor

compatible with increased adrenal salt retaining hormone secretion during the premenstrual phase

The present author's own limited studies on salivary and urinary Na/K ratios in the menstrual cycle have been directed

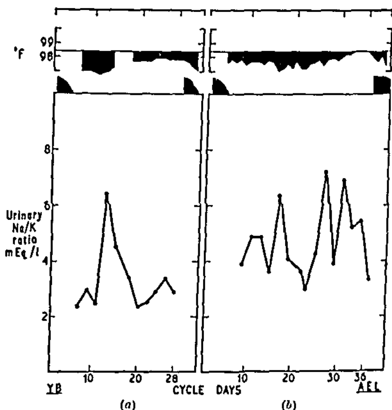


FIG 3 Urinary Na/K ratios in two normal women In (a) there appears to be a peak at about the time of ovulation In (b) the ratio appears to be higher during the second half of the cycle

mainly towards an attempt at elucidating the basis for so called premenstrual tension which is widely supposed to depend upon premenstrual salt and fluid retention (see, for example, Greene and Dalton, 1953, who consider an increased oestradiol/progesterone ratio to be largely responsible) The findings are in agreement with those of Chesley and

Hellman (1957) in that no precise pattern of variation in salivary or urinary sodium and potassium concentrations or Na/K ratios, either in normal women or in those complaining of premenstrual tension, has been discovered

Fig 1 shows two cycles from a normal woman the Na/K

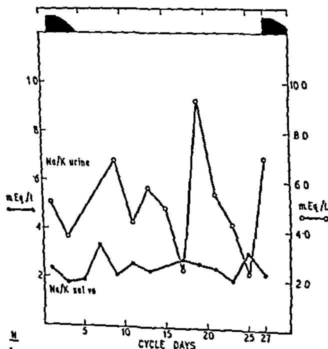


Fig 4 Urinary and salivary Na/K ratios compared in a woman who experienced premenstrual tension

ratio appears to be high at the start of both cycles and there is a distinct fall (mainly due to increased potassium secretion) at what may be judged from the basal temperature record to be the time of ovulation in the second cycle

Fig 2 shows a long but ovular cycle in another normal patient (A E L) No convincing pattern is discernible Fig 3b shows the urinary Na/K ratios in another cycle from

patient A.E.L. If anything, the ratio is higher in the second half of the cycle—i.e. sodium retention is less premenstrually. In Fig. 3*a*, the urinary Na/K ratio appears to rise sharply just at the time of ovulation—i.e. at the time of an oestrogen peak, when, according to the usual view, the tendency should be towards sodium retention.

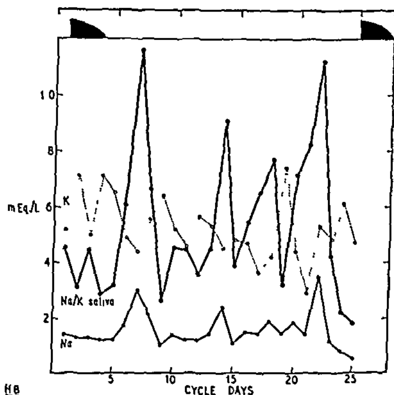
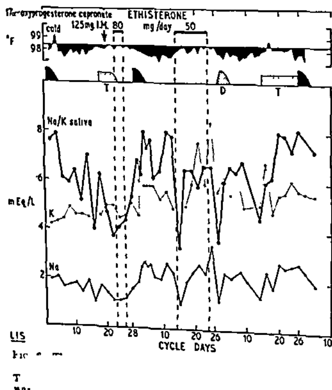


Fig. 5. Salivary sodium and potassium concentrations and ratios in a woman who experienced premenstrual tension.

Figs. 4–6 relate to women who experienced definite premenstrual tension. In Fig. 4 the salivary and urinary Na/K ratios are compared. The latter (note that its scale is ten times that of the salivary Na/K ratio) is much more variable than the former, and neither shows any definite pattern. Certainly there is no evidence of sodium retention premenstrually. Fig. 5 shows the *salivary Na/K ratios in another*

patient; they fluctuate violently but show no evidence of premenstrual sodium retention.

Fig. 6 shows three consecutive cycles in a patient who experienced quite severe premenstrual tension. In the first



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cycle, the Na/K ratio in the saliva was definitely lower, due to a lower sodium concentration, in the second half of the cycle. An injection of 125 mg of 17α oxyprogesterone capronate intramuscularly failed to affect the symptoms, but when ethisterone, 80 mg. daily by mouth, was started three days later the tension disappeared, in spite of the Na/K ratio

patient A.E.L. If anything, the ratio is higher in the second half of the cycle—i.e. sodium retention is less premenstrually. In Fig. 3*a*, the urinary Na/K ratio appears to rise sharply just at the time of ovulation—i.e. at the time of an oestrogen peak, when, according to the usual view, the tendency should be towards sodium retention.

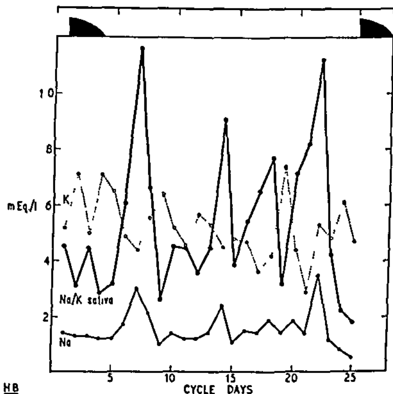


FIG. 5. Salivary sodium and potassium concentrations and ratios in a woman who experienced premenstrual tension.

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quantity during pregnancy has been shown by numerous balance studies (see Rinsler and Rigby, 1957 for references) Chesley and Boog (1943) found an increased thiocyanate space in normal pregnancy, the increase being still greater in pre-eclamptic toxaemia From this it was concluded that much of the sodium retention was due to expansion of the extracellular fluid (ECF) compartment However, Gray and Plentl (1954), using a sodium isotope dilution technique, found little change in the sodium space and total exchange able sodium in normal pregnancy They observed a total gain of some 500 m equiv of sodium during the last six months of pregnancy, which they felt could be accounted for by the products of gestation and the expanded maternal blood volume The maintenance of an essentially unchanged non pregnant sodium space during normal pregnancy, despite the rise in plasma volume, suggests that there is little change in ECF

The gain of sodium and water, with maintenance of a normal total exchangeable sodium value and with an increased thiocyanate space, provides indirect evidence that in normal pregnancy there is an alteration of cell permeability with an increased maternal storage of intracellular sodium and water Th...

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of the F... and plasma volumes It has been demonstrated by Bartter and co workers (1956) that a fall in ECF volume without change in tonicity leads to a rise in aldosterone excretion Such a rise in aldosterone excretion occurs in pregnancy (Venning and Dyrenfurth, 1956, Venning *et al*, 1957 Rinsler and Rigby, 1957) and may form part of a homeost...

one is ... expanded ECF compartment Expansion of this compartment was shown by Bartter and

remaining low *In the next cycle, 50 mg ethisterone daily* was given from the 14th day of the cycle There was no tension (though the succeeding period was painful) Yet again the Na/K ratio appears to have been on the whole lower in the second half of the cycle In the third cycle, no treatment was given, the usual premenstrual tension appeared but this time the premenstrual Na/K ratios were the highest in the cycle

It must be confessed that the writer does not know how to interpret these findings, beyond concluding that they do not provide evidence for theories currently held to account for premenstrual tension and its relief (which in the writer s experience, is by no means invariable) with progesterone or its analogues

Pregnancy

In no physiological circumstances do such profound hormonal changes occur as in pregnancy The output of oestrogens rises some thousandfold of progesterone ten to twentyfold *and of adrenocortical and thyroid hormones to less impressive* but still significantly increased levels A new hormone, chorionic gonadotrophin, found only in pregnancy, of foetal and therefore partly paternal origin—a foreign protein', to *some extent—appears in the circulation immediately after* implantation, rises to striking levels by about the 60th day of gestation and then as rapidly falls to about one quarter the maximum level during the remainder of pregnancy The sum total of these changes is to produce a substantial degree of fluid and sodium retention in all pregnant patients Oedema is of course common, its association with hypertension, with or without albuminuria to give pre eclamptic toxaemia, is also not uncommon Toxaemia is for the obstetrician, one of the remaining major problems he has to face Its pathogenesis continues in obscurity, in spite of extensive research

Only one or two aspects of this vast problem will be dealt with here

That water and sodium are retained in considerable

conclusions, of possible relevance to our main theme, are as follows

Oestrogens In men, the output of oestrogens remains relatively constant with increasing age, in women, on the other hand, the output declines between the ages of 40 and 60 years, reaching a level somewhat below that of men and thereafter remaining constant Of the separate fractions, oestrone and oestradiol decline slowly in men, accompanied by an increase in oestriol which makes the total oestrogen output appear constant, in women the most marked decline in earlier decades is in oestriol output, the least marked in that of oestrone, while in the later decades further small declines in oestrone and oestradiol are accompanied by an apparent increase in oestriol Oestriol is a metabolite, not a secretory product as the other two may be, its increase with advancing age may therefore be due to lesser destruction of secreted oestrogen

Neutral Steroids The rate of decline of 17 ketosteroids is similar in both sexes The urinary ketonic androgens are higher in men than in women and decline more steeply in the former particularly during the earlier decades During these decades the decline of androgens is steeper than that of 17 ketosteroids so that with advancing age the ratio of 17 [illegible] declines more rapidly than that of the less androgenically active 17 ketosteroids Though this might have been expected for men as a result of declining testicular function, it is perhaps more surprising in women and suggests a decrease in output of either adrenal or ovarian androgens, or both

The ratio of androgens to oestrogens is higher for men than for women at all decades until the ninth

The output of adrenal corticosteroids is rather higher in men than in women at all ages and varies but little with age In contrast the non ketonic steroids, a mixture of substances

co workers (1956) to cause a fall in urinary aldosterone excretion in normal persons In the pre eclamptic patients studied by Rinsler and Rigby (1957), the aldosterone outputs were considerably less than those at the same stage of normal pregnancy and it was concluded that this was because of the expanded ECF compartment The output of aldosterone in these toxaemic patients is less, for a given urinary Na/K ratio, than in the normal group, yet despite the low aldo sterone output, sodium retention is maintained or increased This suggests that a mechanism other than that of aldo sterone secretion may be responsible for the sodium retention of pre eclamptic toxaemia

Labour, especially if prolonged, is another aspect of pregnancy in which electrolyte disturbance may assume im portance Hawkins and Nixon (1957) have demonstrated a consistent loss of plasma water and increase in plasma specific gravity after only 20 hours of labour, indicating a state of dehydration long before the appearance of clinical signs In addition, they found an increase in plasma sodium and a decrease in chloride and potassium This, they suggest, is due to increased renal excretion of chlorides necessitated by the disturbance of acid base balance due to ketosis resulting from shortage of available glycogen After 48 hours of labour, a striking fall in plasma potassium and in circulating eosinophils was seen This is consistent with increased adrenocortical activity, such as is known to occur after surgical operations (MacPhee, 1953) In labour, this fall in plasma potassium may be particularly important because of its influence on uterine contraction It is very probable that potassium depletion in long labours materially adds to the inefficiency of an already inert uterus

Changes in Steroid Metabolism in Ageing Men and Women

The most extensive study of this subject has been made by the Worcester group (Pincus *et al*, 1955) Certain of their

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[illegible]
VENNING, E. H., and DYRENFURTH, I. (1956) *J. clin Endocrin. Metab*, 16, 426
VENNING, E. H. [illegible]
WILKINS [illegible]

DISCUSSION

[illegible]

Swyer: No, but I am very interested to hear of these changes.

Scribner: Dr. Swyer, [illegible]

Swyer: No we did not [illegible] nary ambulant persons ar[illegible] It was hoped that if the c[illegible]

of doubtful origin, part adrenocortical and part perhaps gonadal, decline with age much as do the 17-ketosteroids. Thus the outputs of the various classes of neutral steroids change with age in dissimilar fashion. Close study of the data suggests that the steroids of adrenal origin are less affected by age than are those derived from the gonads, but that adrenal steroids are not uniform in behaviour in this respect.

This differential behaviour is clearly shown by the various α-ketosteroids. The *11-deoxy steroids, androsterone* and *aetiocholanolone*, decrease regularly and markedly with advancing age, in both men and women. In contrast, the 11-oxygenated 17-ketosteroids decrease much less markedly with increasing age in both sexes. The 11-oxyaetiocholanolones decrease least of all; these substances derive chiefly from cortisol and its metabolites.

To evaluate the significance for fluid and electrolyte control of these hormonal changes in ageing men and women is none too easy. The most important of the above-mentioned hormones from this point of view are the adrenal corticosteroids, the output of which changes least. Beyond that simple statement it is unsafe to venture.

Nothing has hitherto been said about the rôle of antidiuretic hormone of the posterior pituitary in the control of electrolyte and fluid metabolism under the various circumstances discussed above. Though it is true that numerous reports have appeared in the literature implicating ADH in a variety of pathological states characterized by oliguria and oedema, it is the opinion of van Dyke, Adamsons and Engel (1955) that "the assays used to support this belief are so grossly inaccurate as to make valueless any conclusions that have been reached." If we may accept that opinion, nothing further need be said.

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inappropriate imposition, here the hypotonicity of the extracellular fluid is accompanied by a swelling of the cells An "appropriate" fall in tonicity and increase in volume of the extracellular fluid is not associated with these symptoms The patients I mentioned earlier do not have evidence of water intoxication

After every stress, these patients with hyponatraemia returned to their original low concentration of extracellular fluid One feels that the concentration is determined by the cells—a new steady state We do believe that this low concentration of the extracellular fluid must be the result of a low osmotic pressure of the cells. There are obviously different kinds of hypotonicity of the extracellular fluid, with and without symptoms of water intoxication When there are symptoms, the cells are swollen. Miss Leeson and I have been wondering whether a lack of symptoms means the cells are not swollen, but merely hypotonic.

Sryer I was referring to the opposite problem, namely that in labour dehydration is accompanied by lack of potassium Presumably this increase in specific gravity of the plasma and the apparent loss of plasma potassium would not be consistent with normal functioning of the cells

with people who were keeping themselves on an ordinary kind of régime they would show up in spite of any day-to day variations That is certainly a deficiency in our studies, but I do not think it entirely invalidates them

Thaysen How were the Na/K ratios in the saliva done?

Swyer They were obtained by collecting saliva first thing in the morning, as nearly as possible at the same time each day, for a fixed length of time (five minutes). In one series, the first five minutes was collected, and in another series the first five minutes was discarded and the second five minutes collected, as I understand there is something significant in that We were unable to see any difference at all when done in these two

volume It did not seem to make any difference at all, but I do agree that some of the variables might have been inadequately controlled

Thaysen: I believe that you might find the ratio very reproducible when you use a standard secretory rate

Talbot Dr Swyer, you mentioned something about a will-o'-the-wisp, sodium diuretic hormone of adrenal origin Do you believe in its existence, and if so, have you or any of those here a solid notion as to the nature of the beast?

Swyer: I certainly have no solid notion It is an idea that has been mooted to account for the apparent inability of normal amounts of sodium retaining hormone to counteract the sodium loss I know there have been very active searches for it, and that large amounts are found in the salt losing type of adrenal hyperplasia

Desaulles We have worked quite a lot on this problem and we have got something which is derived from the adrenal, but what it is we *do not* really know Dr. Wettstein (1958 *Iva*, 29, in press) has just described how he found it and how he is working on it, but that is as far as we have got

Adolph I would like to raise a general problem which Dr Swyer brought up How is one to judge whether in labour there is dehydration? All the criteria by which we can judge of the existence of dehy-

McCance: It is a question of definition. Do you mean by dehydration a rise in the tonicity of the extracellular fluid due to an increase in the

[illegible]

and Rowntree, L. G. (1922). *Arch intern. Med*, 29, 306) exemplifies an inappropriate imposition, here the hypotonicity of the extracellular fluid is accompanied by a swelling of the cells. An "appropriate" fall in tonicity and increase in volume of the extracellular fluid is not associated with these [illegible] do not have

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Sayer: High
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and in another series the first five minutes was discarded and the second five minutes collected, as I understand there is something significant in that. We were unable to see any difference at all when done in these two

that the estimation of the Na/K ratio permits one to dispense with the necessity for measuring secretory rate

Swyer Another thing we did was to measure the volumes which were produced in this fixed time, and try to correct for the variations in volume It did not seem to make any difference at all, but I do agree that some of the variables might have been inadequately controlled

Thaysen I believe that you might find the ratio very reproducible when you use a standard secretory rate

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retain water before birth and still excretes high amounts of oestrogens after birth, that the persisting influence of the mother's oestrogens in the early days of life might be the explanation of the infant's poor response to a water load.

Adolph This oestrogenic influence seems to me a very interesting possibility. Has anyone any data on the influence of the maternal hormones upon water balances or exchanges?

Fourman Another question is whether oestrogens do inhibit the [illegible]

[illegible] handling of the water load since that extends for the best part of the first year, or so I understand

McCance No, only about 14 days, I believe

Adolph I think I can clarify this contradiction of ages to some extent If you read the literature up to 1923 you learn that in the first year the [illegible]

[illegible] as great, and within 90 days even as great as in the adult human, if one bases water load and excretion on unit body weight.

Swyer Does this also apply to resistance to dehydration and handling of electrolytes?

Adolph I do not think we have any good data on the resistance to dehydration We know much less about hydropaenia than we do about superhydration

[illegible]

[illegible]

Young: I think there is a much simpler explanation for the young infant's rapid rise in serum potassium under conditions of stress The [illegible]

dration. I have no real proof of this, but all neonates becoming dehydrated very quickly show a high serum potassium level.

Milne In these cases in babies with high serum potassium, is the myocardium less sensitive to the hyperkalaemia? This could be inferred from the work of Widdowson and McCance (1956. *Clin Sci*, 15, 361) on serum potassium in foetal pigs Anyone with experience of hyperkalaemia in acute renal failure in adults would find very severe ECG changes long before the serum potassium reached 10 m equiv./l, and death usually occurs very shortly after the potassium reaches 10 m equiv /l These high figures rather startle me, I would like to know what is happening to the ECG during the period of hyperkalaemia

Davson The effect of potassium on the heart is linked with that of calcium It may be that over long periods the calcium might rise too and tend to compensate for the raised potassium.

Scribner We have made some studies on dogs and we could not greatly increase the tolerance of the dog to hyperkalaemia by giving calcium Large doses of calcium increased tolerance no more than 1 m equiv./l

[illegible]

young males, it is a very frightening experience to see the heart misbehaving with both the clinical effects and the ECG changes of hyperkalaemia.

McCance: You seem to have found something in which the infant [illegible]

of infants, sometimes the physiologist's point of view has made the [illegible]

logy are borne in mind

There is one point I should like to make which refers to the papers by Prof Adolph and Dr Swyer It seems to me, since the baby tends to

GENERAL DISCUSSION

Richet Dr Thaysen mercury poisoning is supposed to inhibit [illegible]

substance?

Thaysen I have not done any experiments of this kind myself, [illegible]

Richet Dr Desaulles has reminded me that during chronic mercuric poisoning acrodynia for instance, there is an increase in sweating

Thaysen That might be due to a cerebral effect of chronic mercury poisoning rather than to a local effect of the mercury directly on the glands

Dawson It is rather a fortunate accident that the mercurials are diuretics and that they have that specific action on the kidney [illegible]

[illegible] parallel increase in the plasma magnesium, cellular magnesium also went up but rather less

Richet We have made determinations of plasma magnesium in more than 200 patients during acute and chronic renal failure During acute renal failure there is always an increase in plasma magnesium concentration Our technique with yellow titanium gives normal values of 1 5–1 7 m equiv /l In acute renal failure we sometimes get 3 0–3 5 m-equiv /l serum magnesium In contrast, serum

tends to delay the development of serious dehydration during the neonatal period This process coupled with other attributes might enable some newborn infants to survive total thirsting as long as an adult

Heller: I seem to remember that what Gans and Thompson showed was that there was a decrease of body water in the infant which was correlated with the excretion of maternal oestrogens, but this does not establish a causal relationship

[illegible]

to the other

Wallace Dr Swyer, what about the situation of a diabetic woman and her baby? In a great number of instances there is a very intense water retention

Swyer: I can counter that by saying what about the baby of a prediabetic mother? It shows just the same changes before the mother has diabetes I do not think we know why the prediabetic mother has a large baby—there have been suggestions that it is due to excess growth hormone secretion by the mother, but there is no very convincing evidence

Wallace This kind of baby generally seems to have a great deal of water in him—more water than in equivalent weight normal babies

Swyer That is very true The baby is large but it is not postmature—indeed, it behaves more like a premature

Wallace Is that an oestrogen effect?

Swyer I do not think we know

Wallace Very often during these discussions the words "inefficient" and "immature" have been used to describe the newborn infant Mr Peter Rickham in his book, "The Metabolic Response to Neonatal Surgery" (1957 Harvard University Press), develops the point of view that the newborn infant is tolerant of adverse experiences such as fasting, thirsting and surgical trauma Despite the fact that the newborn has an extra load of water in his body and a low metabolic rate he does seem to have a certain toughness that at a later date is not so evident "Immaturity" and "inefficiency" may not be synonymous

[illegible]

and salt management that we are talking about today—we failed to find any evidence that the small children react any worse than their [illegible] Surg, [illegible] large [illegible] least [illegible] older children and adults

Dawson Was there also a control on whether salts were being absorbed when they say that five time isotonicity would have stopped it? Just the fact that the skin absorbs water does not mean that salts are not absorbed as well

skin by a layer of air The type of salt used determines the amount of water vapour in the air The results by this technique agree with the hypertonic solution studies

Hingerty What salts have been investigated?

Scribner Sucrose potassium chloride sodium chloride The phenomenon is believed to be purely an osmotic effect

Borst Before the war Viennese clinicians reported on considerable absorption of water by the skin in heart failure The *prognosis* could even be determined by studying the rate of absorption Dutch

potassium is increased in only 20 per cent of our patients We have

potassium remains normal for a long time

McCance That agrees with observations Miss Watchorn and I made in 1932 (*Biochem J*, *26*, *54*) *We generally found that the* serum magnesium was high in chronic renal failure and indeed searched for such cases when we wanted high values for our ultra filtration experiments

Scribner I want to bring to your attention the work done by Dr Konrad Buettner, professor in the Division of Climatology at the University of Washington, Seattle (1953 *J appl Physiol*, 6, 229) His observations bear on the sweating data that we have heard and also on considerations of cellular tonicity If you study water

trations of sodium chloride solution, the skin will take up water until a concentration which is five times isotonic is reached The mechanism of absorption is not known and there has been no work to elucidate why this occurs The rate of absorption in an adult human is about 20 ml /hr for the total skin, and is correspondingly less for

low rates of sweating, data on electrolytes in sweat may be abnormally high throughout due to this absorption, and there is some chance that by the proper control of conditions you may be able to absorb water in survival experiments at sea, since sea water is only three times isotonic

Dawson What happens to the water? Is it immediately carried away by the capillaries?

Scribner Yes Deuterium studies have shown that Ten—twenty ml /m^2/hr are the actual figures for the absorption

Talbot In the last war in survival ration studies we immersed some very dehydrated volunteer subjects in the equivalent of sea

Dawson Was there also a control on whether salts were being absorbed, when they say that five time isotonicity would have stopped it? Just the fact that the skin absorbs water does not mean that salts are not absorbed as well

BODY WATER COMPARTMENTS THROUGHOUT THE LIFESPAN*

H. VICTOR PARKER, KNUD H. OLESEN, JAMES McMURREY and BENT FRIIS HANSEN

Surgical Service and Laboratories of the Peter Bent Brigham Hospital, Harvard Medical School, Boston, and Queen Louise's Children's Hospital, Copenhagen

OUR first knowledge of the composition of the body was acquired during the last decades of the nineteenth century. The methods used were desiccation and chemical analysis which allowed the determination of the contents of water and electrolytes in carcasses or in single organs. With the recent introduction of the dilution methods a new field of study has grown up based on the *in vivo* measurements of the total quantities of body water and its partitions. Direct dilution methods are now available for the measurement of total body water and of the extracellular water. The intracellular water is calculated as the difference between total body water and extracellular water and is thus a derived value (Moore *et al.*, 1956).

A few comments should be made about the methods and the evaluation of the measurements. In the material presented the total body water has been determined as the volume of dilution of deuterium oxide. In the children the extracellular water has been measured as the volume of dilution of thiosulphate and in the adult groups as the volume of distribution of radioactive bromide corrected for red cell bromide, for the relative water contents of plasma and interstitial water, and for the Donnan effect. As the volume of dilution of thio

* This work was supported by a grant from the United States Atomic Energy Commission to the Peter Bent Brigham Hospital (AT-(30-1)-733).

sulphate is smaller than the corrected volume of dilution of radiobromide the values for extracellular volumes will not be directly comparable for the children and the adults The same will apply to the calculated intracellular water All the methods used are reproducible within the 5 per cent range As the absolute quantities measured are difficult to compare from one individual to another it has become customary to express the results as relative values The standard of reference used is the body weight as this standard in our experience has been the most simple In the interpretation it is important to realize that a rather large biological variation appears within groups of the same age and sex

Although the study of the body water compartments throughout the lifespan is still fragmentary, certain trends in relation to age and sex have appeared It will be the purpose of this paper to outline these features in a description of the body water compartments during the three main phases of life growth maturity and ageing

Growth

Growth implies a variety of fundamental processes cell multiplication increase in cell size accumulation of extracellular material increase in fat and minerals

The alterations in the body water compartments during growth have been studied by Friis Hansen (1956) From a series of 93 normal children studied with deuterium oxide with thiosulphate or with both a series of 31 individuals with simultaneous measurements of all three water compartments will be presented

It appears from Table I that the absolute amounts of total body water of extracellular water and of intracellular water demonstrate an increase throughout infancy and childhood It is seen that the intracellular water rises more markedly than the extracellular water

In Table II the three measurements are given as percentages of body weight The total body water shows a relative decrease throughout infancy and childhood with a most marked

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Within the body water compartments the measurements with thiosulphate demonstrated a relative decrease of extracellular water during growth. A similar degree of decrement in extracellular space with advancing age has been reported by Ely and Sutow (1952) using the thiocyanate method, and by Cheek (1954) using the corrected bromide space. The relative values for intracellular water in the series presented stayed about the same throughout infancy and childhood. No similar investigations are available in the literature, but it is interesting that Corsa and co workers (1956) found that the total exchangeable potassium as related to body weight stayed the same throughout infancy and childhood. As about 95–98 per cent of the exchangeable potassium must be present within the cells their results can be taken as corroborative evidence for Friis Hansen's (1956) findings of the relative constancy of the intracellular water.

An alteration in the interrelationship between the extracellular and intracellular water during growth thus appears. When the extracellular water is expressed as a percentage of total body water the extracellular compartment decreases from 55 per cent in the youngest group to 38 per cent and 28 per cent in the two oldest groups again reflecting the relative decrease of the extracellular water. This altered relationship between the extra and intracellular water is another important facet in the body compositional changes *during growth*.

The alterations during growth could be produced in two ways (1) They could be due to a proportional alterat [illegible]

Histochemical studies are helpful in the interpretation of this problem. Kerpel Fronius (1937) found in studies of muscular tissue from human *newborn* babies and from adults a relative increase in intracellular phase during growth, whereas such a change did not appear in the skin or in the central nervous tissue. Kerpel Fronius also drew attention to the fact that the total muscle water had increased from

decrease during the first two years of life The relative decrease in extracellular water is more marked than the decrease in total body water The intracellular water demonstrates about the same relative value throughout childhood It should be mentioned that no sex difference appeared in this

Table I

BODY WATER COMPARTMENTS IN CHILDREN ABSOLUTE VALUES

Age	*Water compartments in litres* *TBW*	*ECW*	*ICW*	*Number of subjects*
0–11 days	2 65	1 45	1 20	5
11–180	3 10	1 42	1 68	9
½–2 years	5 40	2 36	3 04	7
2–7	8 96	3 40	5 56	9
7–14	27 62	7 52	20 10	1

series The tendencies found in this group are similar to the findings in the larger group including cases with single measurements of total body water or of extracellular volume A statistical analysis of the larger group has shown that most of the differences between the age groups are significant

Table II

BODY WATER COMPARTMENTS IN CHILDREN RELATIVE VALUES

Age	*Values in per cent of body weight* *TBW*	*ECW*	*ICW*	*Number of subjects*
0–11 days	76 4	41 6	34 8	5
11–180	72 8	34 9	37 9	9
½–2 years	62 2	27 5	34 7	7
2–7	65 5	25 6	39 9	9
7–14	64 2	17 5	46 7	1

The relative decrease in total body water with advancing age indicates a relative increase in total body solids, i e cell solids, mineral solids and body fat The total body solids thus represent the fraction of the body which demonstrates the highest degree of absolute increase during growth This increase in total body solids represents one important facet in the alterations in body composition with advancing age

for the relative water contents of plasma and interstitial water, and for the Donnan effect. As the extracellular water according to the method applied here shows a higher normal value than is obtained with the thiosulphate method the results for extracellular and intracellular water in this series will not be directly comparable to the findings in the group of children.

Table III

Body water compartments in adults. Absolute values

Sex	Age range	Body weight kg	Water compartments in litres TBW	ECW	ICW
Males	23–54	72.5	38.9	16.8	22.1
Females	23–51	59.3	28.7	13.3	15.4

The absolute average values for total body water, extracellular water and intracellular water appear in Table III. As expected all values are higher in the males than in the females, corresponding to the higher average weight in the male group. Most of the difference in total body water is accounted for by the difference in intracellular water.

Table IV

Body water compartments in adults. Relative values

Sex	Age	Weight kg	Water compartments in per cent of body weight with standard error of the mean TBW	ECW	ICW
Males	23–54	72.5	54.3	23.4	30.9
			±1.39	±0.64	±0.89
Females	23–51	59.3	48.6	22.7	25.9
			±1.47	±0.54	±0.96

In Table IV the average values are given in per cent of body weight. The males contain 54.3 per cent of total body water whereas the females contain 48.6 per cent. This difference is statistically significant ($P=0.01$). The relative values for the extracellular water are very close to one another. The intracellular water amounts to 30.9 per cent in the males and to 25.9 per cent in the females. This difference is statistically significant ($0.01 > P > 0.001$).

29 per cent of total body water in the newborn baby to 51 per cent of total body water in the adult and he stressed that an *increase in total muscular tissue rich in intracellular phase* was a prominent feature in the alterations in body composition during growth

Yannet and Darrow (1938) found in their studies of cats a relative increase in intracellular phase during growth in muscles, whereas only very small alterations appeared in liver tissue or in brain tissue. In studies of growing chickens Barlow and Manery (1954) reported a similar relative increase in the intracellular phase in muscular tissue.

It appears from these studies that the alterations measured with the dilution methods must be results of a development varying quantitatively and qualitatively from one tissue to another.

In conclusion the alterations in body composition during growth can be described as a disproportional increase in total body solids, total body water, extracellular water, and intracellular water When the values are related to body weight the following trends are seen during growth a decrease in total body water, an increase in total body solids, a decrease in extracellular water, and a relative constancy in intracellular water When the water compartments are related to total body water the trend is for a relative decrease in extracellular water and a relative increase in intracellular water

Maturity

The body water compartments in adults will be described with particular reference to the sex difference

The material presented comprises ten normal males and ten normal females at ages from 23 to 54 years, average age in the middle thirties The series was studied by H V Parker in Dr Francis D Moore's laboratory, Peter Bent Brigham Hospital, Boston (McMurrey *et al*, 1958) The methods applied were total body water was determined with deuterium oxide, the extracellular water was measured as the radiobromide space, which was corrected for red cell bromide,

It appears from the series that males have a higher relative content of body water than females, confirming the results with the deuterium oxide method reported by F... co workers (1950 ...

be ... relative amounts of extracellular water in the series presented The extracellular water represented 22 7 per cent of body weight in the females and 23·4 per cent in the males This similarity in the relative values for extracellular water is in ... Ch...

... bromide space, of ... Ikkos and Luft (1957) using the thiosulphate method, and of Griffin and co workers (1945) using the thiocyanate method.

The lower relative content of total body water in females as compared to males in the series presented is due to a relatively lower content of intracellular water in the females. A similar difference in the content of intracellular water appears in the series studied by Ljunggren, Ikkos and Luft (1957) in which the intracellular water was calculated on the basis of an extracellular space measured with radiobromide as well as with thiosulphate Further evidence of the relatively lower content of intracellular water in females compared to males is present in the consistent findings of a lo... amount of total exchang... by Edelman and co ... Solomon (1954), Bla... (1954), Sagild (1956), and Ljunggren, Ikkos and Luft (1957)

The lower relative body water in females and exten...

... values for intracellular water and total exchangeable potassium, must be assumed to be lower in females than in males if ... a higher ... than ma...

The similarity of the relative values for the extracellular water and dissimilarity of the relative intracellular water volumes in the two sexes gains further support from other parts of the same study As is seen in Table V, simultaneous studies of total exchangeable sodium and potassium were *carried out in these patients according to the method des* cribed by Moore and co workers (1956) The total exchange able sodium which was determined through an independent measurement demonstrates relative values very similar in the two sexes As about 85 per cent of the total exchangeable sodium can be accounted for in the extracellular space the findings can be taken as supportive evidence for the correct ness of the very close relative values for the extracellular

Table V

BODY WATER COMPARTMENTS AND TOTAL EXCHANGEABLE ELECTROLYTES IN ADULTS

Sex	*Values related to body weight with standard error of the mean*				
	ECW (%)	Cl_e (*m equiv /kg*)	Na_e (*m equiv /kg*)	*ICW* (%)	K_e (*m equiv /kg*)
Males	23 4	29 3	39 5	30 9	48 0
	±0 64	±0 71	±1 06	±0 89	±1 38
Females	22 7	28 6	38 3	25 9	39 4
	±0 54	±0 92	±1 09	±0 96	±1 10

water in the two sexes The relative values for the total exchangeable potassium which was determined independently of the intracellular water demonstrate a pattern very similar to the findings of the intracellular water In both measure ments the females have a relative value about 20 per cent *below the males* As 97 per cent of the total exchangeable potassium must be within the cells this finding can be taken as evidence for the correctness of the measurements of the intracellular water It is worth mentioning that a calculation of the average intracellular potassium concentration in the two sexes results *in very similar values 152 m equiv per* litre intracellular water in the males and 149 m equiv per litre intracellular water in the females, and thus indicates that no difference in cellular composition exists in the two sexes

and from 25 9 per cent to 22 4 per cent in females The differences mentioned are not statistically significant except for the decrease in intracellular water in males ($0.01 > P > 0.001$)

The tendency to a decrease in the relative values for total body water found in both sexes is mostly due to a decrease in intracellular water From an unpublished study of Dr N W Shock (1957) in which the antipyrine space and the thiocyanate space were measured in a larger group of males, the following data are of interest A comparison of 23 subjects

Table VI

Body water compartments in younger and in older adults relative values

Sex (Number)	Age	Weight kg	Water compartments in per cent of body weight with standard error of the mean TBW	ECW	ICW
Males	23–51	72 5	54 3	23 4	30 9
(10)			±1 33	±0 64	±0 89
Males	71–84	63 1	50 8	25 4	25 4
(7)			±1 55	±1 36	±0 58
Females	23–51	59 3	48 6	22 7	25 9
(10)			±1 47	±0 54	±0 96
Females	61 74	63 0	43 1	21 4	22 4
(7)			±1 32	±0 45	±0 97

aged 40–49 and 32 subjects aged 70–79 showed that the values for total body water related to body weight decreased from 54 8 per cent to 50 9 per cent, and those for the calculated intracellular water decreased from 30 5 per cent to 25 1 per cent The extracellular water changed from 24 3 per cent to 25 8 per cent only The same pattern of a slight decrease in total body water and in intracellular water related to body weight was seen in a male series studied by Olbrich and Woodford Williams (1956) Sagild s findings of a decrease in total exchangeable potassium in the old age groups of both sexes can also be interpreted as evidence of a decrease in the intracellular phase related to body weight (Sagild 1956)

When the body water compartments are related to total body water as a standard of reference another sex difference appears. In males the extracellular water accounts for 43 per cent of total body water and in females for 47 per cent, whereas the intracellular water amounts to 57 per cent of total body water in the males and 53 per cent in the females. The difference between these ratios is statistically significant ($P<0{\cdot}001$). This difference in the distribution of the total body water between the extracellular and intracellular compartments can be explained as the result of a higher development of tissues rich in intracellular material and relatively poor in extracellular phase, such as muscle tissue, in the males.

In conclusion the sex difference in body composition is outlined as a higher relative content of total body water, a higher relative content of intracellular water and a lower relative amount of total body solids and especially of body fat, in males than in females. The total body water is distributed with a lower extracellular fraction and a higher intracellular fraction in males than in females.

Ageing

Our experiences in the old age group are based upon the investigations carried out in seven apparently normal males with an average age of 75 years and seven apparently normal females with an average age of 68 years. This group was studied in Dr Francis Moore's laboratory (Parker, Olesen and Moore, 1958). The methods used were the same as those applied to the younger adults.

The essential findings in the old age group are presented in Table VI.

A comparison between younger and older adults reveals the following findings: total body water decreases from 54·3 per cent to 50·8 per cent in males and from 48·6 per cent to 43·4 per cent in females. The extracellular water rises slightly in males and decreases slightly in females. The intracellular water decreases from 30·0 per cent to 25·4 per cent in males

and from 25·9 per cent to 22·4 per cent in females. The differences mentioned are not statistically significant except for the decrease in intracellular water in males ($0{\cdot}01 > P > 0{\cdot}001$).

The tendency to a decrease in the relative values for total [illegible]

Shock (1957), in which the antipyrme space [illegible] cyanate space were measured in a larger group of males, the following data are of interest. A comparison of 23 subjects

Table VI

BODY WATER COMPARTMENTS IN YOUNGER AND IN OLDER ADULTS RELATIVE VALUES

Sex (Number)	*Age*	*Weight kg*	*Water compartments in per cent of body weight with standard error of the mean* TBW	ECW	ICW
Males	23–54	72 5	54 3	23 4	30 9
(18)			±1 49	±0 64	±0 89
Males	71–84	68 1	50 8	25 4	25·4
(7)			±1 55	±1 36	±0 58
Females	23–51	59 3	48 6	22 7	25 9
(10)			±1 47	±0 54	±0 96
Females	61 74	63 8	43 4	21 4	22 4
(7)			±1 32	±0 45	±0 97

aged 40–49 and 32 subjects aged 70–79 showed that the values for total body water related to body weight decreased from 54 8 per cent to 50 9 per cent, and those for the calculated intracellular water decreased from 30·5 per cent to 25 1 per cent. The extracellular water changed from 24·3 per cent to 25 8 per cent only. The same pattern of a slight decrease in total body water and in intracellular water related to body weight was seen in a male series studied by Olbrich and Woodford-Williams (1956). Sagild's findings of a decrease in total exchangeable potassium in the old age groups of both sexes can also be interpreted as evidence of a decrease in the intracellular phase related to body weight (Sagild, 1956)

From the uniform tendencies in these materials it seems reasonable to conclude that the slight decrease in total body water and in intracellular water related to body weight reflects real alterations in the body composition with advancing age With the decrease in the relative value for total body water there is a relative increase in total body solids As the intracellular phase shows a relative decrease the increase in total body solids must be assumed to be caused by a relative increase in non cellular solids, most probably body fat

The alterations in the extracellular water related to body weight are not quite uniform and the changes are small It is of interest that extracellular water expressed as per cent of total body water in both sexes shows a rise from younger to older subjects, in the males from 43 per cent to 50 per cent, in the females from 47 per cent to 49 per cent This tendency is also seen in Shock's and in Olbrich and Woodford Williams series and indicates an altered relationship between the extracellular and intracellular water

In conclusion the alterations in body composition in the old age group as compared to younger adults were rather small A tendency to a relative decrease in total body water and in intracellular water and a relative increase in total body solids, most probably body fat, was found The extracellular water stayed essentially the same in values related to body weight, but demonstrated a tendency to increase in per cent of total body water

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DISCUSSION

Haggerty Are these differences in the intracellular water related to the proportion of functional muscular tissue? Have ...

fat ...
reg ...

Olesen It appears from Dr Iris Hansen's material that chemical maturity occurs about the age of twelve months

Widdowson Have you made any calculations of the body fat at different ages?

Olesen I have tried to compare the different groups and it seems that there is a relative increase in body fat throughout childhood It is a slight one but it does exist if we accept that all the non cellular solid changes are changes in body fat This calculation is quite apart from possible changes in body minerals and I do not know to what extent *these would interfere*

Borst Is there any relationship between the creatinine output and the intracellular fluid?

[illegible] of determination of [illegible] orators (Corsa *et al* [illegible] was found between creatinine excretion and the amount of total exchangeable potassium This has not been studied in this particular series

Heller How far is it justifiable to take mean figures from ten young adult females without considering the role of the menstrual cycle? Have you had enough cases to pay attention to this point?

Olesen No but it would appear from what Dr Swyer mentioned yesterday that it would not mean very much as the latest view is that these body weight changes are randomly distributed throughout the menstrual cycle

Shock It seems to me that we have two possible interpretations of this age reduction in intracellular water The interpretation I favour is that the reduction in total intracellular water is a reflection of the loss of functional cells or the loss of protoplasm rather than a change in the water concentration of the remaining protoplasm Have we any other evidence that would make one interpretation more probable than the other?

Dawson I think that is a very sound point because a cell can change in size *without there being a change in the relative value of the* water or solid contents of the organism Is there any change in the histological appearance of old tissue that would indicate whether the cells had become smaller or larger?

[illegible]

for the age reduction in *basal metabolism in terms of cellular mass in body* [illegible] al metab [illegible] ygen con [illegible]

surface area

Scribner Total exchangeable potassium might possibly be a good parameter for this measurement *of protoplasm*

interesting study (to be
n either body

for instance, to the blood volume,

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wasted and has not much
toplasm than in fat.
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THE EFFECT OF VARIABLE PROTEIN AND MINERAL INTAKE UPON THE BODY COMPOSITION OF THE GROWING ANIMAL *

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THE quantities of various nutritive substances in the growing body at any given point represent the metabolic integration of the daily additions to the body from the diet from the time of conception. Measurement of the rate or quantity of addition may or may not measure the nutritional requirement for a given substance. Whether it does or not will depend upon the requirement for synthesis and metabolic transformation and upon the possibility of the body being able to store the substance. Thus, the day-by-day accretion of fat or glycogen cannot measure a requirement but the accretion of protein and mineral may do so, once any capacity for storage is exceeded. Information concerning requirements for growth is usually obtained by measurements of external balance for variable periods of time The information acquired concerning the requirements for growth and the composition of growth by this method is often strangely contradictory and always incomplete Much of the data so obtained indicate that extensive storage of dietary components occurs, or that the composition of the body tissues is variable and dependent upon quantity and quality of the intake.

* This work was supported by grants from the Baker Laboratories, Inc., Cleveland, Ohio and the National Institute of Arthritis and Metabolic Diseases [illegible] Health Service, [illegible] Society, May [illegible]

That body tissues can vary significantly in composition except under extreme conditions is difficult to reconcile with present-day knowledge of tissue composition

The experiments to be described here were undertaken in an attempt to characterize the effects of high and low mineral and protein intakes, in various combinations, upon the body composition of the growing albino rat as determined by direct whole body analysis Previous work using this method of approach has been concerned with single constituents and not with the interrelationships of all of the components The data indicate little variability in composition for the collective soft tissues of the body The only intake-dependent relationship that seems of significance is in the relative proportions of skeleton to soft tissues

Experimental Methods

A Animals and Diets

Male weanling Sprague Dawley strain rats were used in all feeding experiments Two groups of animals were used to measure food consumption on the high and low protein diets In these experiments spill proof feeding tunnels were used, and the animals caged singly The remaining groups of animals were housed in units of four in steel wire cages with open mesh bottoms Continuous access to unlimited quantities of food in open containers was allowed Distilled water was similarly offered from dropping bottles All groups of animals were allowed to grow for a period of 20–25 days This period of time was chosen as it allowed approximate doubling of weight for the most slowly growing groups

The experimental diets were compounded using powdered fat free cow's milk (Starlac, The Borden Company), electrolyte and vitamin free casein (Nutritional Biochemicals Corporation, Cleveland) dextrose a fat mixture composed of equal parts of corn oil (Mazola Corn Oil, Corn Products Refining Co, Argo, Illinois) and hydrogenated vegetable oil (Crisco, Proctor and Gamble, Cincinnati, Ohio), and a salt

mixture ($NaHCO_3$, 7.4 g, KCl, 12.0 g, $CaCO_3$, 12.0 g, $(NH_4)_2HPO_4$, 14.9 g, $MgSO_4$, 2.5 g, KI, 0.001 g) to produce the compositions shown in Table I. The salt mixture was compounded to imitate the ion ratios found in fat free cow's milk. Ferrous sulphate, 2.0 g, copper sulphate 0.22 g and aureomycin 0.25 g per kg of diet were incorporated in the mixtures. A vitamin mixture (Vitamin Diet Fortification, Nutritional Biochemicals Corporation) in quantities calculated to make all diets equal in this respect was added to the mixtures.

Table I

ANALYSIS OF DIETS

Diet	g/100 g Diet *Protein*	*Fat*	*Carbohydrate*	*Ash*	*Other**	mmole/100 g Diet *Na*	*K*	*Cl*	*Ca*	*P*
HPHE	23.4	30.0	35.5	6.02	5.1	16.00	32.4	28.3	23.1	21.3
HPLE	23.4	30.0	35.5	3.08	5.1	8.04	16.0	14.5	11.8	10.9
LPHE	12.0	32.0	50.5	6.02	2.4	16.00	32.4	28.4	23.1	21.3
LPLE	12.0	32.0	50.5	3.08	2.4	8.04	16.0	14.5	11.8	10.9
Friskies	26.8	6.5	51.4	11.00	3.[illegible]	15.10	15.3	13.0	84.0	50.8

* Moisture + Fibre (calculated by difference)

Prior to the beginning of the feeding experiments all animals had been weaned to a commercially produced small animal feed (Friskies, The Carnation Milk Company) known to produce excellent growth, general health and reproduction in the albino rat. Preliminary feeding trials with the high protein experimental diets in comparison with the Friskie diet indicated equal effectiveness as measured by weight gain, general appearance, activity, gentleness and lack of morbidity.

Eight groups of animals were studied, namely

1. Weanling group (WEAN) 70–80 g rats weaned to *Friskies*
2. High Protein High Electrolyte (HPHE) see Table I
3. High Protein Low Electrolyte (HPLE) see Table I
4. Low Protein–High Electrolyte (LPHE) see Table I
5. Low Protein–Low Electrolyte (LPLE) see Table I

6 Rats fed Friskies by way of control See Table I for composition of this ration
7 A high protein, high electrolyte group fed to measure food consumption
8 A similar group to No 7 but fed the low protein, low electrolyte diet

At the end of the allotted period of growth (20–25 days) the animals were etherized and 2 ml of blood removed for analysis either by heart puncture or tail incision Killing was accomplished by further ether exposure The dead weight was obtained and the abdominal cavity, thorax and skull opened with heavy shears * The whole body was then dried in an oven at 85° 95° C until a constant weight was reached (4–5 days) During the drying process, the carcass was further broken up with heavy shears The disintegrated carcass was extracted repeatedly with a cold mixture of equal parts ethyl and petroleum ether and re dried to constant weight The dried extracted carcass was then homogenized in a Waring Blendor with 5 volumes of anhydrous acetone and the solvent evaporated off and the material re dried This process produces a fine homogeneous powder suitable for quantitative analysis The powder was stored in a desiccator

B Chemical Methods

Water Calculated from weight loss after desiccation

Fat During the course of the analytical work, the fat extraction method used as applied to tissues by Hastings and Eichelberger (1937) was examined for completeness of fat extraction when applied to whole carcass Powdered carcass was exhaustively extracted in the Soxhlet apparatus serially using ether alcohol and chloroform This process increased the degree of fat extraction to the extent of 1 5–4 g per animal Analysis of the material subjected to such extraction

* Intestinal contents were not [illegible]

indicated that its nitrogen content multiplied by 6 25 plus the weight of its ash very closely approximated 100 per cent of the material Consequently, fat has been calculated in all of the data by the relation Fat=dead weight−water weight−(nitrogen×6 25+ash weight) All of the constituents shown in Table II have been calculated as g, m mole or m equiv per 100 g of protein plus ash (i e fat free dry solids)

Ash A sample of carcass powder was weighed after incineration at 600° in platinum

Nitrogen Determined by macro Kjeldahl analysis using selenium as a catalyst

Chloride A micro modification of the method of Lowry and Hastings (1942) was used with cold nitric acid filtrates Samples of the homogenized powder were also analysed polarographically for chloride, using sulphuric acid filtrates with excellent agreement between the two methods

Sodium, Potassium and *Calcium* These were determined on the ash after separation of calcium using methods previously described (Bergstrom and Wallace, 1954)

Magnesium Determinations were done on the ash using the method of Fister (1950)

Phosphorus This was determined on the ash by the method of Fiske and Subbarow (1925)

All electrolyte and nitrogen analyses were in duplicate

Results

The analytical data obtained in the experiments are shown in Table II For comparative purposes the whole body analyses on the albino rat of Light and co workers (1934) and of Cheek and West (1956) are included Also shown are the average data of Widdowson and Spray (1951*B*) for six normal human newborn babies and the data for single whole adult human bodies of Widdowson, McCance and Spray (1951*A*) Forbes Cooper and Mitchell (1953) and Mitchell and co workers (1945) The data for water, protein and ash have been calculated per kilogram of fat free body weight The water and electrolytes are also shown using as a reference standard

Group	RATS Wean	HHHE	HPLE	LPHE	LPLE	Friskies	Light 1934	Cheek 1936	1951B	1951A		
No. of Animals	10	8	12	12	8	10	7	(2)	6	1 (L)	1	1
Body Wt. g. S D	71 4 ±3 39	170 6 ±7 18	171 6 ±11 4	111 9 ±8 95	121 8 ±17 1	165 4 ±8 92	274 6 (1)	166 0 (2)	3500 0 (4)	45 0 kg	53 0 kg	70 55 kg
Fat Free (F F) Body Wt (5) S. D	64 9 ±3 18	151 8 ±7 28	149 7 ±10 05	97 7 ±7 15	102 9 ±12 37	151 0 ±9 04	230 0 (1)	150 0 (2)	2930 0	34 4 kg	42 8 kg	61 42 kg
H_2O g/kg F F Body Wt (5) S D	795 0 ±0 68	762 0 ±2 39	774 0 ±0 78	771 0 ±0 72	755 0 ±1 95	766 0 ±0 49	734 0 (1)	756 0 (2)	821 0 (4)	732 0	604 0	779 0
Protein g/kg F F Body Wt. (5) S D	170 7 ±6 00	202 2 ±10 3	194 3 ±7 21	185 5 ±7 02	203 0 ±15 4	192 5 ±5 2	224 5 (1)	203 4 (2)	148 0 (4)	102 0	235 3	165 0
Ash g/kg. F F Body Wt (5) S D	34 6 ±1 11	33 2 ±5 8	31 1 ±0 88	40 9 ±3 0	40 9 ±4 8	41 0 ±0 85	40 6 (1)	41 1 (2)	31 2 (4)	75 8	68 6	55 0
H_2O/100 g. Prot + Ash S D	387 0 ±15 8	323 0 ±42 4	344 0 ±14 6	342 0 ±15 0	312 0 ±31 5	328 0 ±0 87	277 0 (1)	309 0 (2)	458 0 (4)	273 5	220 0	353 0
Na m-equiv /100 g Prot + Ash S D	26 2 ±0 66	20 9 ±1 22	22 2 ±0 80	24 1 ±1 72	23 2 ±1 56	24 6 ±0 17	21 3 (1)	21 4 (2)	55 0 (4)	48 2	—	—
K m equiv /100 g Prot + Ash S. D	35 0 ±0 69	30 0 ±1 21	35 9 ±1 30	34 9 ±1 10	33 4 ±0 61	33 7 ±0 67	22 0 (1)	29 3 (2)	28 4 (4)	36 5	—	—
Ca m equiv /100 g. Prot + Ash S D	215 0 ±11 5	109 0 ±20 5	167 0 ±9 5	252 0 ±21 0	222 0 ±19 8	243 0 ±7 6	238 0 (1)	215 0 (2)	270 0 (4)	620 0	306 0	415 0
Mg m-equiv /100 g Prot + Ash S D	15 1 ±0 55	11 1 ±0 73	11 9 ±0 82	14 0 ±0 82	12 8 ±0 95	14 1 ±1 49	9 8 (1)	11 7 (2)	12 2 (4)	9 3	—	—
Cl m-equiv /100 g Prot + Ash S D	21 5 ±0 61	16 6 ±0 50	18 2 ±0 70	18 5 ±0 90	18 9 ±1 23	17 6 ±1 05	13 6 (1)	15 9 (2)	—	—	—	—
P m mole/100 g Prot + Ash S D	95 0 ±2 40	82 0 ±5 9	77 7 ±2 95	96 3 ±7 72	92 9 ±3 89	85 4 ±4 1	95 5 (1)	—	98 6 (4)	208 6	124 2	129 0
Na Serum m-equiv /l S. D	—	143 8 ±3 03	143 7 ±2 49	143 4 ±5 08	146 0 ±3 26	150 2 ±4 7	133 7 (1)	146 0 (3)				
K Serum m-equiv /l S D	—	6 6 ±0 73	5 3 ±0 51	6 2 ±0 42	5 8 ±0 50	6 77 ±0 57	6 11 (1)	4 7 (3)				
Cl Serum m-equiv /l S D	—	97 2 ±2 39	104 1 ±2 42	104 5 ±2 71	99 8 ±3 06	99 8 ±2 31	102 2 (1)	110 0 (3)				
Serum Prot g /l S. D.	—	5 61 ±0 18	6 14 ±0 22	5 29 ±0 26	5 09 ±0 34	6 39 ±0 45	6 28 (1)	—				

(1) See original data for min. and max values.
(2) Derived from regression equations.
(3) Mean data only 70–410 g animals.
(4) No measure of variation available
(5) Calculated as ash plus protein plus water
Data of Cheek and of Widdowson calculated on basis of fat free dry solids. All other whole body data calculated as m-equiv, m mole, or g per 100 g protein plus ash (see text)

100 g of protein plus ash This is equivalent to the commonly used reference standard of fat free dry tissue (*vide supra*)

Fig 1 graphically presents the currently obtained data in terms of grams of ash, protein and water per kilogram of fat free body The grams of fat per kilogram of fat free body are shown to the right of the columns It is evident that the compositions of the fat free bodies are essentially similar The relative proportions of water, ash and protein have not been greatly modified by variation of the diet producing the growth

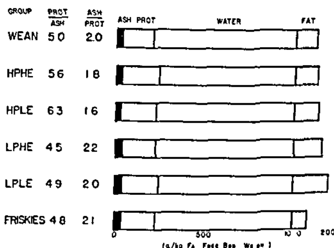


Fig 1 Ash protein and water content calculated per kilogram of body weight for the six groups Fat per kilogram of fat free body is shown at the right

increment Only if body fat were included would gross variation occur The young animals (WEAN) are relatively low in ash and protein and high in water with growth the bodies acquired relatively more ash and protein than they did water The fat contents of the animals on the low protein diets are significantly higher than they are on the high protein

In Fig 2 the absolute values for total fat free body weight, water, protein and ash for the five groups are shown as contrasted against the Friskie group as an arbitrary reference

standard of growth The high protein groups are very closely equivalent in weight, protein and water content to the standard The two low protein groups reach two thirds of the high protein groups with regard to weight, water and protein The degree of mineral accretion in the high protein animals is significantly different, the high electrolyte group accreting

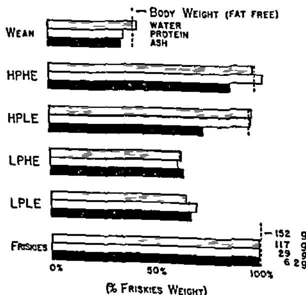


FIG 2 [illegible]

much more than the low, but less than the Frisk [illegible]

[illegible] the gain of ash is not significantl [illegible]

[illegible] animals show a greater relative and absolute accretion of protein than do the low

protein, high electrolyte group. This is significant at the 1 per cent level.

The protein to ash ratios shown in Fig. 1 and evident in Fig. 2 indicate the main significance for body composition resulting from diets of variable protein and electrolyte content. The high-protein-fed animals have more protein in relation to

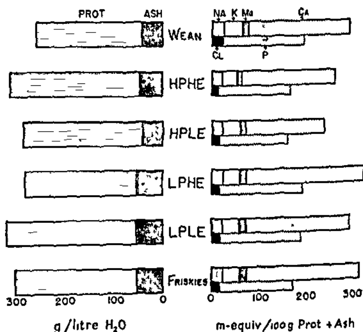


Fig. [illegible] on the left is of the ash
and [illegible]
On t [illegible]
their [illegible]

ash than do the low-protein-fed animals. Since bone contributes 90 per cent of the ash, the ratios represent the soft tissue to bone proportions in a very general yet valid way. It seems evident that only on a high protein intake can the growing body lay down maximal bony tissue. In the Friskie group where the ash of the intake is very high and composed chiefly of calcium salts, an even greater accumulation of ash

occurs at the relative expense of soft tissue Where this relationship stops is not answered by the present data

tion of ash and protein in the body water and the nature of the composition of the ash are shown in Fig 3 It is evident, as has been noted that only in the weanlings and in the low protein high electrolyte group does a significantly different amount of protein per unit of water appear

All of the experimental data for individual constituents of the body have been calculated using four reference para meters i e grams or m mole per whole body, per kilogram of fat free whole body, per kilogram of water and per 100 g of protein plus ash (fat free dry tissue) All of these calculated individual values have been compared among the four groups The following statements can be made

I The Effects on the Protein Content of the Body

A By Protein Intake

Only in those animals on the high electrolyte diets did increased protein intake result in increased protein content of the body on any of the enumerated bases

B By Electrolyte Intake

In the animals on the high protein intakes, the electrolyte effect was variable depending upon the reference base used for calculation In the low protein fed animals a high electro lyte intake reduced the protein content of the body calculated on any basis

II The Effects on the Mineral Content of the Body.

A By Protein Intake

On any basis of calculation, other than absolute body size, the bodies of the animals fed a low protein intake, whether with high or low electrolyte, contained more ash, calcium,

magnesium, sodium, chloride and phosphorus than those fed a high protein intake.

B. By Electrolyte Intake.

The high electrolyte diets led to increased calcium and decreased chloride in all groups calculated on any basis.

In the high protein groups the high electrolyte intakes also resulted in more ash and less potassium when calculated on any basis

In Table II the serum concentrations of sodium, potassium, chloride and total protein are shown for the four experimental groups. The only consistent significant difference is for the concentration of total serum protein Serum protein concentrations are higher in the high-protein-fed groups The lower protein concentration may indicate protein deficiency in the low protein group and other evidence for such deficiency is given below. The validity of serum protein concentrations as a reliable index of protein malnutrition can be questioned. In this connexion it is of interest that the serum protein concentration of the breastfed infant is lower than that of the infant fed cow's milk (Tudvad, Birch-Andersen and Marmer, 1957).

Animals in experimental groups No 7 and No. 8 were fed in such a manner as to allow accurate measurement of food intake. The high protein group consumed 8 2 g of ration per animal per day in contrast to 9 3 g per day for the low protein group. The mean weights for the two groups at the end of 23 days were 174 and 155 g respectively. Calculation of the caloric values for the whole bodies of these animals shows that the high protein group contained 292 calories per average animal (1,710 calories per kg) and the low protein group 263 calories per average animal (2,085 calories per kg). Calculation of the calories utilized for physiological activity indicates that the low protein group expended 175 calories more per animal for the period of observation than did the high protein group Increased spontaneous activity was clearly evident in the low protein groups during the period of

observation. Increased spontaneous activity with nutritional deficiency has been previously noted (Forbes *et al.*, 1935; Bevan *et al.*, 1950)

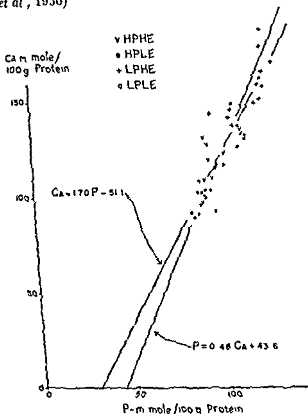


FIG. 4 Relationships of calcium and phosphorus to protein in the experimental groups For description of method of construction, see text

The data in Fig. 4 represent the calcium/phosphorus relationship in the four principal experimental groups. On the assumption that the protein content is a basic unit of structure, the values are compared in relation to protein. One advantage of this formulation is that the intercept of the

regression line on the X axis defines the amount of phosphorus present in 100 g of calcium free protein This value should reflect primarily the phosphorus content of muscle tissue From the statistical analysis of the calcium phosphorus relationship, a correlation coefficient of + 0 90 was derived Further, by the analysis of variance technique, it has been determined that the regression curve is a straight line, described by the equations calcium = 1 70 phosphorus − 51 1 and phosphorus = 0 48 calcium + 43 6 when both are expressed as m mole/100 g protein, and calcium = 2 19 phosphorus − 2 04 and phosphorus = 0 37 calcium + 1 35 when both calcium and phosphorus are expressed as g /100 g protein The X intercept is between 30 3 and 43 6 m mole phosphorus/100 g protein or between 0 93 and 1 35 g phosphorus/100 g protein It is of interest that the calcium/phosphorus ratios of the four groups of rats studied by Light and co workers (1934) and the infants analysed by Widdowson and Spray (1951) also lie on this regression line when their values are calculated in this manner This indicates that the changes in phosphorus content of the various groups are related to the changes in calcium and to the total amount of protein present The phosphorus concentration is constant in the "soft tissue" (calcium free protein), and the phosphorus *has a constant ratio to the calcium in the "skeleton"* (calcium containing tissue)

It is also apparent from the figure that the calcium to protein ratio is highest in the low protein high electrolyte fed animals and lowest in the high protein low electrolyte group

Discussion

The present data, like the very similar data of Widdowson and McCance (1957) and Stanier (1957), indicate no real evidence for storage or depletion of protein with varying intake The basis for such a judgment is made by examination of data calculated using either a kilogram of fat free whole body or 100 g of fat free dry solids as a standard of reference

The rationale for the use of the latter standard has been discussed in detail elsewhere (Cotlove *et al*, 1951) While such a reference point is essential for evaluation of acute shifts of water and electrolytes in tissues, it may not be equally applicable where the growth of a complex of tissues is involved In this latter situation it is essential that the relative gain or loss of a substance in question be examined in regard to a number of reference standards, as has been done here (see Results) When the change in any constituent is consistent in direction regardless of the reference basis, it is probably a real one, as has been noted above However, when the change is in one direction on one basis and in the opposite on another, the question of gain or loss is difficult to assess An example of this from the current data is found in the change in potassium content with change in protein intake in the animals on the low electrolyte diets The high protein fed animals were larger and contained more potassium on an absolute basis When calculated per kilogram of fat free body the potassium concentrations were equal, but on a litre of water basis the potassium was greater in the low protein group Again, referring this ion to fat free dry solids, the high protein fed animals would seem to have the highest content For the purposes of nutritional evaluation, it is valid to calculate constituents as per unit of whole body inclusive of fat When this is done, an even greater number of permutations and combinations can be found with regard to relative contents of all substances Until more is known concerning the distribution function and relationships of protein and electrolytes in tissues it would seem advisable to emphasize only those changes which are relatively consistent

When the present data are considered on this basis, the composition of the body with regard to water, protein and ash is the same despite variation of the components of the intake The whole body may be smaller or larger as limited by the availability of certain crucial nutriments but its relative composition remains unchanged Only the relative size of the skeletal mass in relation to soft tissue seems to be significantly

susceptible to some variation by variation of dietary intake Even in relation to skeletal tissue the possibility of variable composition is limited by another parameter, i e protein Thus, the composition of the body achieves an independence from the environment, an independence that would seem essential in a living system where metabolic function is carried on by protein with its critical requirement for constancy of water and ionic concentration

The concept that the whole body or the cells of the body may be enriched or depleted of their various chemical constituents by variation of the dietary intake is widely supported in the nutritional literature By examination of retentions during balance observations on growing infants, it may be concluded that the higher the intake of a substance, the greater will be its final concentration in the body per unit of weight (Rominger and Meyer, 1927, Swanson and Iob, 1933, Stearns, 1939)

Correlation of weight gains of premature infants with the protein and ash content of the milk fed has shown high positive correlation with the increasing ash content (Kagan *et al*, 1955) Conversely, possible support for the concept of variable body composition stems from nitrogen losses after trauma Both animals and men maintained on low protein intakes lose less nitrogen after trauma than do those with prior optimal intakes (Munro and Cuthbertson 1943, Cuthbertson 1948) Holmes, Jones and Stanier (1954) found evidence indicating that men shifted from very low protein intakes to optimal intakes retained nitrogen far in excess of that calculated from weight gain and external losses The use of the terms ' depletion' and deficiency" bears tacit evidence for the belief in the concept of cellular impoverishment during nutritional deprivation The majority of the evidence for the concept of variable storage of protein and minerals and loss during deprivation stems from the technically hazardous techniques involving measurement of external balances The possibility of low correlation between apparent retentions or losses and changes in body weight has not been

commonly realized The shortcomings of the balance method are functions of such items as the effects of variable caloric intake, quality and quantity of protein intake and mineral ratios on the fat content of the body, the distribution of body water and the relative size of body components such as skeleton and muscle These problems have been most completely explored in relation to evaluation of the problem of protein adequacy (Mitchell, 1944, Allison, 1954, Calloway and Spector, 1953, Spector and Calloway, 1953) It is also little appreciated that systematic errors occur in the calculation of apparent retentions that are cumulative in a positive direction, the magnitude of the cumulative error being in direct proportion to the magnitude of the intake This makes difficult the comparison of retentions at variable intakes The relevance of this criticism with regard to calcium retentions has been discussed by Mitchell and Curzon (1939) and by Mitchell and co workers (1945)

Examination of the composition of growth increments by direct body analysis has shown that, once chemical maturity is reached, the composition of the fat free body with regard to protein and ash is nearly constant regardless of any procedures taken to modify weight gain (Moulton, 1923 Moulton [illegible]

[illegible] of calcium (Light *et al.*, 1934)

The concept of variable cellular composition of the body is difficult to reconcile with the knowledge of the composition of tissues All of the individual tissues of the albino rat have been analysed for their water, protein, fat and mineral content by many investigators All of these data show a monotonous constancy when calculated on a fat free basis This occurs despite almost infinite variation in the rations fed to the animals Unless special experimental [illegible]

impo[illegible]

comp[illegible]

et al [illegible]

[illegible] composition is constant Even

with age the maximum change of water content is no more than 1 per cent and of potassium 5 per cent

Examination of whole body data, with certain salient exceptions, also shows rather remarkable constancy Fat is probably the only component of the total body that can vary within rather wide limits and still allow reasonable well being to exist Variation from 10 to 50 per cent can occur without apparent evidence of malfunction The water content of the fat free body is more closely guarded Variation of much more than ± 5 per cent from a rather rigid norm results in rapid increments of physiological disability Moreover, allowable variation of body water is primarily extracellular, cellular water content, within the limits of viability, must be confined to much smaller variations Since protein is the critical parameter against which water content must be judged, it follows that protein concentration must also be highly critical and susceptible to only minute variation The consideration applying to water must also hold for the chief extracellular electrolytes, sodium and chloride Deficit of potassium in the whole body to the extent of approximately 25 per cent does occur, and is replaced by variable gains of total body sodium (Schwartz Cohen and Wallace, 1953, Cheek and West 1956) The studies of Sherman and Booher (1931) show that the calcium content of the whole body is widely variable in response to variation in the dietary intake Definition of the optimal body content of this ion is elusive

In the discussion so far the point of view has been taken that in order to justify the terms *stored protein* or *mineral*, these must exist as physically demonstrable entities comparable to glycogen and fat in the body It would appear that the essential organic structure of the body cannot be affected in quality by adjustment of the diet The careful chemical analyses by Luck (1936) of rat liver proteins from animals maintained on varying levels of protein intake indicate that all fractions of the liver proteins have participated equally in any "storage' process Madden and Whipple (1940) have defined the reserve store of protein as all of the protein

which may be given up by an organ or tissue under uniform conditions without interfering with organ or body functioning This definition indicates primary physiological significance not anatomical In this view the primary requirement for furthering understanding would be methods for characterizing and distinguishing physiological depletion The response to repletion has been used to assess the degree of depletion in such a physiological sense The work of Madden and Whipple (1940) and Cannon (1954) illustrates the fruitfulness of the method for studying the metabolism of protein under conditions of deficit Cooke and co workers (1952) and Schwartz, Cohen and Wallace (1955) have applied the technique to experimental potassium deficiency and Hansen (1956) to the potassium deficit in kwashiorkor The ability to survive in stressful situations provides a further avenue of approach Baur and Filer (1957) employing the weanling pig growing on diets similar to those used in the present experiments have shown differing abilities of animals growing on different diets to resist water and caloric deprivation Their data indicate that animals maintained on low protein intakes survive caloric deprivation to a greater degree than do those maintained on high protein intakes Conversely, the high protein fed animals withstand water deprivation to a greater degree than do their low protein fed companions Sherman (1946) has correlated calcium intake with life span and reproductive life A newly opened approach to the problem of characterizing and assessing deficits in a physiological sense is that of distinguishing structural versus enzyme protein in tissues Potter and Klug (1947) have shown that liver octonoate and succinate oxidases are depressed ...

... number of other tissue enzymes

Summary and Conclusions

The composition of growth of the albino rat on high protein high electrolyte, on high protein low electrolyte, on low

protein high electrolyte and on low protein low electrolyte diets has been examined Analysis of the whole body for protein, water, fat, ash, sodium, potassium, chloride, calcium phosphorus and magnesium was performed on animals allowed to double their weaning weights on the enumerated diets

The animals on the low protein intakes grew significantly less and their bodies contained more fat The composition of the fat free bodies on a unit basis were all essentially similar despite the variation of the food intake The principle difference resulting from variation in intake was in the quantity of the skeletal constituents in the various groups The animals consuming the low protein rations contained more calcium and phosphorus on a unit basis than did the high protein fed animals

On the high protein intakes accretion of skeletal minerals was dependent upon the level of electrolyte intake, being higher in the high electrolyte fed animals In the low protein fed animals accretion of skeletal minerals was less affected by the level of electrolyte intake

Only in the animals on the high electrolyte diets did increased protein intake result in increased protein content of the body

The significance of the data for nutritional evaluation is discussed

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DISCUSSION

Widdowson: May I suggest, Prof. Wallace, that you started your experiments far too late. If you had started at 21 "Adolph days" instead of 21 "Wallace days", you might possibly have got different

larly the skeletal muscle, is more rapid in the fast-growing rats.

Wallace How are they different? Are they dilute?

Widdowson The proportion of extracellular fluid in the bodies and muscles of all the rats decreases with development, and the proportion of intracellular constituents, nitrogen and potassium, rises, but the changes take place more quickly in the fast-growing animals, so that they reach chemical maturity at an earlier age

Ann appl Biol, 13, 374, and 14, 171) He did fantastic things like suckling mice with rat foster-mothers and getting them up within 21 days to something like 75 per cent of an adult mouse's weight I went over this again, breaking the changes down week by week (1957, *J.*

to body weight, so naturally the bigger rat ate more and continued to grow faster.

some would get a higher protein and electrolyte intake than others.

[illegible]

regard If gain of weight per gram of protein consumed is calculated the values are 0 41 g per g gain and 0 30 g per g gain for the high and low [illegible]

[illegible] of the low protein diet

Wallace [illegible]
find out
are the [illegible]

extrem[illegible] conditions which do change body compos[illegible]
I do not believe that
by changing the pla[illegible]
more accurately the
by the balance met[illegible]

McCance What would be the effect of change in diet on electrolytes in the body? Our conclusion at the moment is that it has little effect on the composition of the cell

Wallace [illegible]
change the [illegible]
but its comp[illegible]

Heller [illegible]
of the diet [illegible]
We have rec[illegible]
plantains, [illegible]

After about four weeks there was an increase of 5–7 per cent in total body water, but the interesting thing is that the plasma potassium and plasma [illegible]

Wallace: The calcium in the body is almost entirely skeletal and with this kind of data it is impossible to say just where this calcium is. You have to study the individual tissues.

Fourman: Do you think that the increase in bone which you suggested took place is an increase in trabecular bone—so called freely available, mobilizable, bone tissue?

Wallace: [illegible] trabecular [illegible] electrolyte [illegible] conditions of stress

McCance: You began by putting up charts of balances showing that if the diet contained more sodium and potassium, the child absorbed and retained more. Yet you find by experiment that you do not alter the composition of the body. Can you reconcile those observations?

Wallace: This is a purely technical matter on which I have strong feelings. In a balance experiment the quantity of food entering the body and the excreta recovered are always slightly less than the measurements indicate. The more refined the technique the smaller this error is. Also, the greater the concentration of a nutriment in the intake the greater will be the error when compared with intakes of lower concentration but of equivalent caloric value. When subtraction is used to calculate the balance these errors accumulate. The errors in doing a balance are not randomly plus or minus as is generally believed, but systematically positive. Much of the arithmetical difficulty arises because one must subtract two quite large numbers to obtain the usually very small balance value. At zero intake the balance method becomes more accurate. Body composition estimates such as can be made from Benedict's and Gamble's fasting data agree with direct analysis data quite well. However, body composition estimates made from balance data [illegible]

THE EFFECT OF AGE ON THE BODY'S TOLERANCE FOR FASTING, THIRSTING AND FOR OVERLOADING WITH WATER AND CERTAIN ELECTROLYTES *

NATHAN B TALBOT and ROBERT RICHIE

Department of Pediatrics Harvard Medical School and the Children's Medical Service Massachusetts General Hospital Boston

As is well known, the body is equipped with homeostatic systems designed to maintain water and electrolyte content and concentration values at physiologically optimal levels The systems accomplish this task largely by equating output with input While rates of input can be varied widely without overreaching the capacities of the homeostatic systems concerned nonetheless there are limits beyond which one cannot go without getting into difficulty (Talbot, Crawford and Butler 1953, Talbot *et al*, 1955) Thus for each substance there is a *physiological minimum requirement* or *floor*, which is the least intake of the substance in question needed to balance output and hence to prevent deficits where conservation forces are acting maximally There is also for each substance a *physiological maximum tolerance or ceiling* which is defined as the largest amount of the substance that can be taken and eliminated without seriously disturbing body composition Rates falling between these two parameters may be said to fall within the *physiological* or *safe working range* When the rate of administration of a substance falls outside this range for an appreciable length of time, body composition deviates from normal and manifestations of disordered homeostasis develop as outlined in Table I

* This paper is based on work supported by grant A-803 of the National Institute of Arthritis and Metabolic Disease by grants H-1529 and HTS 5139 of the National Heart Institute United States Public Health Service and by a grant from the Commonwealth Fund of New York.

The manner in which a limit to homeostatic capacity can be recognized and defined is illustrated in Fig. 1 (Talbot *et al.*, 1956). Here it can be seen that this patient maintained a normal potassium status, as judged from electrocardiographic T waves and from serum potassium concentration, and remained in potassium balance at rates of intake up to approximately 70 m-equiv. per m.² per day. These rates of

Table I

INDICATIONS THAT INTAKE IS PHYSIOLOGICALLY EXCESSIVE OR INSUFFICIENT (ADULT VALUES)

Substance	*Too Much*	*Too Little*
H_2O	Water intoxication Serum water >3.8 ml./m-osm.*	Hypohydration Serum water <3.4 ml./m-osm.*
Na	Extracellular oedema Na_E ↑ > 20%	Extracellular dehydration Na_E ↓ > 12%
K	Weakness, ECG T waves ↑ Serum K >6.5 m-equiv./l.	Weakness, ECG T waves ↓ K_I ↓ >20%
P	Serum P >6 mg %	Osteomalacia

Na_E = extracellular sodium K_I = intracellular potassium
* Corrected for urea.

intake could therefore be considered to be within his safe working range. By contrast, higher rates of intake led to a sustained positive balance and to the appearance of elevated T waves and hyperkalaemia, which are taken to be signs of potassium intoxication. Accordingly, it may be said that this individual's ceiling of tolerance for potassium was about 70 m-equiv. per m.² per 24 hours, a subnormally low value in comparison with a normal ceiling of at least 250 m-equiv. per m.² and in keeping with the fact that he was suffering from marked impairment of renal function.

The same principles have been used in estimating the upper and lower limits of body tolerance for water and certain electrolytes for normal individuals of various ages, depicted in Fig 2 The upper limits shown in this figure are of necessity approximate, being based on the relatively few data available in the literature and the files of our metabolic unit (Talbot *et al*, 1952, Talbot, Crawford and Butler, 1953, Talbot *et al*,

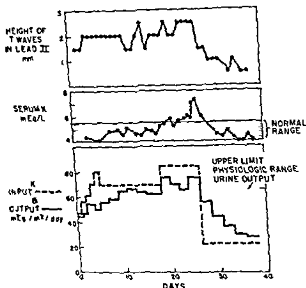


FIG 1 Demonstration of physiological maximum tolerance for potassium in a patient with impaired kidneys. (*From* Talbot *et al* 1956)

1955, 1956, Talbot, Richie and Crawford, 1958) In all instances, they are intended to represent levels which can be attained by healthy individuals within a day or so and not the uttermost levels which can be attained after extensive prior conditioning The lower limits include normal growth requirements for infants and children, a factor of relatively small size after the first few months of life It can be seen that with the exception of young infants, individuals normally

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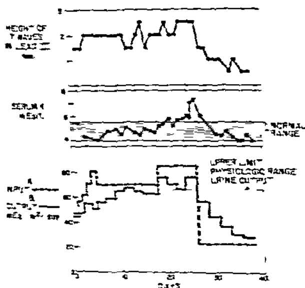


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Na_E = extracellular sodium. K_I = intracellular potassium.
* Corrected for urea.

intake could therefore be considered to be within his safe working range. By contrast, higher rates of intake led to a sustained positive balance and to the appearance of elevated T waves and hyperkalaemia, which are taken to be signs of *potassium intoxication*. Accordingly, it may be said that this individual's ceiling of tolerance for potassium was about 70 m equiv. per m.² per 24 hours, a subnormally low value in comparison with a normal ceiling of at least 250 m equiv. per m.² and in keeping with the fact that he was suffering from marked *impairment of renal function*.

which occur during the growth period, in each case average normal values for body composition and content were used (Shohl, 1939, Forbes and Perley, 1951, Corsa *et al*, 1956, Friis Hansen, 1957) Each substance has been considered separately In dealing with water, sodium and potassium,

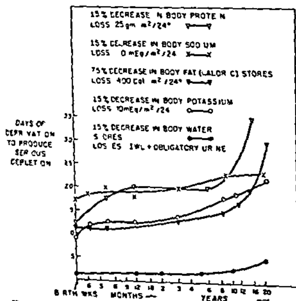


FIG 3. Days of deprivation (ordinate) needed to produce the percentage decrease in body content indicated for each substance in individuals of various ages (abscissa) The rates of loss indicated for each substance approximate to physiological minimum output rates of which some are indicated by the lower boundaries of the physiological tolerance ranges shown in Fig 2

rate of loss was taken as the physiological minimum requirement value indicated in Fig 2 In considering body fat (calorie) stores, energy expenditures were assumed to be at the rate of 1,800 calories per m ² per day (Macy, 1942) and to be derived entirely from body fat Body protein losses were calculated assuming a basal rate of loss amounting to 25 g per m ² per day, the minimum value attained by individuals

utilize but a small segment of their homeostatic capacities. In early infancy, the margins of safety are relatively quite narrow, a fact long recognized by those interested in paediatrics.

The clinical significance of these homeostatic parameters

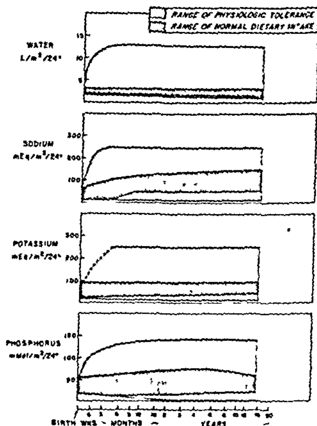


Fig. 2. Estimates of the safe working ranges of intake for individuals of various ages and of the portions of these ranges used by persons taking ordinary diets for age.

may be visualized by considering the length of time needed for individuals of various ages to lose a significant portion of their body stores when totally deprived of water or certain other substances (Fig. 3). In calculating these time values, attention has been given to the changes in body composition

water, +7 per cent (Wynn, 1956), potassium, +5 per cent (Drescher *et al*, 1958), total body sodium (euproteinaemic subjects), +30 per cent (Leaf, personal communication) In the case of phosphorus the end point chosen was elevation of extracellular inorganic phosphorus concentration to 12 mg

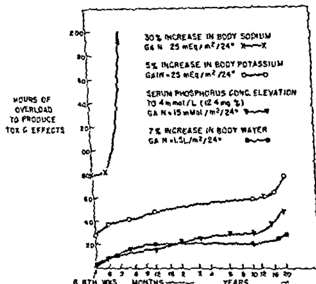


FIG 4 Hours of overload (ordinate) needed to produce the percentage increase in body content indicated for each substance in individuals of various ages (abscissa) The rate of gain is that which obtains when rate of input exceeds the physiological maximum tolerance levels for adults shown in Fig 2 by approximately ten per cent

per cent * Individuals who have surpluses of these degrees are apt to show the signs of intoxication listed in Table I

As might be expected, Fig 4 indicates that infants are relatively much more vulnerable to overloading than older children and adults This is true not only in the relative terms depicted here, but also in absolute terms because the quantity needed to produce intoxication in a small individual

* This assumes no bodily capacity for cellular or skeletal storage of surplus inorganic phosphorus a point on which we have no objective information.

receiving at least 75 grams of carbohydrate per m.^{2} per day (Gamble, 1946–7). It was arbitrarily decided that a 15 per cent decrease in body water, sodium, potassium or protein or a 75 per cent depletion of body fat (caloric) stores constituted a significant and potentially serious loss.

As indicated by the upward trend from left to right of the curves of Fig. 3, infants and children up to three years of age, when deprived of any one of the substances represented, are apt to become depleted two to four times faster than adults. For example, infants will develop as serious a degree of water depletion within one and a half days as adults do in the course of about five days of total thirsting. Likewise, infants deprived of electrolytes or protein or calories may lose an appreciable portion of their body stores of these items after nine to 17 days of deprivation.* By contrast, it takes 20 to 35 days for adults to become similarly depleted under conditions where homeostatic conservation forces are operating efficiently. These observations indicate that in infants who must be maintained by parenteral fluid therapy for more than a few days, special attention should be given to the provision not only of water, carbohydrate and the main extracellular and intracellular electrolytes, but also of maintenance allotments of calories and either preformed protein or amino acids. The same would apply to older children and adults who are depleted or have to be sustained by parenteral fluid therapy for more than a week or ten days.

Fig. 4 deals with the opposite phenomenon of overloading. Here again it has been necessary to make arbitrary decisions concerning the size of the overload and the degree of retention to be considered significant. It was decided to postulate rates of input that were ten per cent in excess of *adult* physiological maximum tolerance or ceiling values. The end point values for the retentions of toxic degree resulting from these physiologically excessive rates are related to the respective average normal body content values at each age as follows total body

* The rate of loss would be considerably greater under conditions of zero carbohydrate intake (Gamble, 1946–7).

dextrose, 40 m-equiv. of sodium, 35 m equiv. of potassium, 40 m-equiv. of chloride, 20 m-equiv. of lactate and 15 m-equiv. of phosphate (Talbot, Crawford and Butler, 1953; Talbot *et al*, 1955). The first set of subjects received their allotment by mouth in an essentially continuous (hourly dose) manner, the

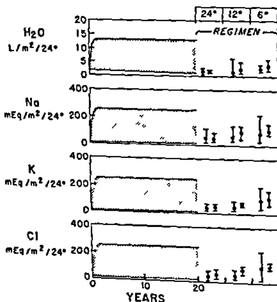


FIG 6 Relations between rates of output observed for subjects on [illegible] [illegible] Neyzi, Bailey and Talbot, 1958)

second set at twice the rate for 12 hours each day and the third set at quadruple the rate for six hours out of every 24. As indicated by the length of the vertical lines at the right of Fig 6, those on the 24-hour regimen utilized but a small fraction of their physiological ranges of excretory capacity in accomplishing metabolic homeostasis. By contrast, those on

is not very great The curves indicate that one is apt to become water and phosphorus intoxicated before one becomes potassium or sodium intoxicated It is interesting that these relations are in keeping with clinical observations on patients with marked limitation of renal function (Talbot *et al*, 1956)

One of the areas where the foregoing considerations appear to have practical implications is with respect to parenteral fluid maintenance therapy Review of hospital practices

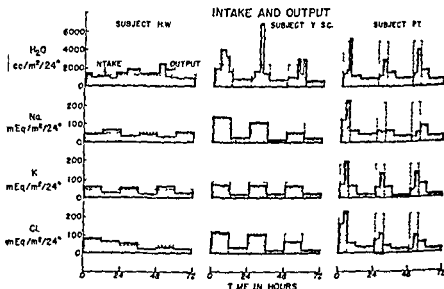


FIG 5 Intake and output of water and electrolytes by normal adult subjects receiving a standard maintenance allotment of multiple electrolyte plus dextrose solution in 24 12 or 6 hours each day (From Neyzi Bailey and Talbot 1958)

reveals that some physicians give the total daily fluid, carbohydrate and electrolyte allotment in a slow continuous manner while others administer the total daily dose in a few hours, allowing the patient to fast and thirst for the remainder of the 24 hour period The data shown in the right hand sections of Fig 5 (Neyzi, Bailey and Talbot, 1958) indicate the ranges of output rate observed on two sets of three normal adults maintained for three days on an ordinary dose (1,200 ml per m ² per day) of a solution containing per litre, 50 g of

may encounter in young infants and in patients undergoing the stress of anaesthesia and surgery As the columns show, the percentage gains to be expected for infants are approximately twice as great as those to be expected for adults While the gains indicated for adults are borderline as regards

approximate terms the limits of capacity of the body to adjust output of water and certain other substances in accordance with homeostatic needs, and to illustrate the clinical implications of such knowledge

These thoughts are presented in the hope that they may elicit constructive suggestions concerning these highly significant, yet rather elusive phenomena

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Homeostasis

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the 12 hour and especially those on the six-hour regimens used almost fully their normal adult ranges of renal excretory adjustment in the course of each 24 hour period When the homeostatic adjustments in water and electrolyte excretion exhibited by these adult subjects are viewed with relation to the infant ranges of homeostatic adjustment indicated by the shaded zones of the left-hand sections of Fig 5, it can be seen

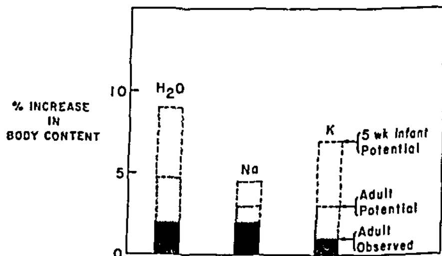


FIG 7 Percentage increases in body water sodium and potassium content

that they are considerably greater than those of which such young individuals are capable

Fig. 7 depicts the percentage increases in body water, sodium and potassium content which would occur during the course of the infusion period if a day's total maintenance allotment of 1,500 ml per m² per 24 hours * were administered in six hours to a patient who was unable to increase rates of urinary output above the physiologically low levels characteristic of fasting and thirsting, a situation which one

* This is an ordinary allotment for infants and children on our Service.

parenterally. It is said that one of the safeguards in oral ingestion of water is the fact that elimination goes on about as fast as absorption

Talbot As far as water, sodium and potassium are concerned, it is six one way and half a dozen the other whether they are taken by vein or by

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DISCUSSION

Black There seems to be some conflict between Dr Talbot, who says that large intakes should produce retention and Prof Wallace who tells us that large intakes produce large arithmetical errors In this matter I am on Dr Talbot's side, and that is not entirely the emotional reaction of someone who has done a certain amount of balance experiments I think we have some supporting evidence in that if balance experiments are done on an adult person who has just had an operation and is on a milk intake (in which the errors of measurement should be much the same as those of excreta), there is quite a definite correlation between intake and retention (Davies, H E F, Jepson, R P and Black D A K (1956) *Clin Sci*, 15, 61)

Bull What was the nature of the load imposed in the experiments on the tolerance of loading?

Talbot The rate of intake of the substances in question is increased in a stepwise manner which allows time for compensatory homeostatic adjustment in rate of output to take place At each step measurements are made to find out whether the body content and/or concentration of the substance is being kept within physiological limits by appropriate adjustments of the rate of output As rate of input is increased it eventually reaches a point where the body is unable to keep its content and concentration values within normal limits by suitable adjustment of rate of output This point is considered to be the upper limit of physiological tolerance or physiological ceiling for the substance in question Rates of input in excess of this ceiling level produce a tendency to abnormal retention For example in the case of potassium when the rate of input exceeds the physiological ceiling value body potassium content increases above normals levels and hyperkalaemia develops together with signs of potassium intoxication

McCance I would like a firm definition of what you mean by tolerance and capacity to eliminate De Wardener did some experiments in which he took large amounts of water every day for 7 or 14 days and although he did not succumb and appeared to tolerate them perfectly well there were finite changes in his responses sensitivities etc (de Wardener

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and refine them more by detailed definitions of certain important interrelations

You are correct in your deductions concerning our aims in defining physiological key variables The present definitions are of necessity approximate and potentially subject to modification and refinement At the same time they are proving to be of value as indices of patient status and as a point of departure for investigation

observed that rats offered gradually increasing quantities of potassium in their diet ate and absorbed the relatively very large quantities needed to produce a lethal degree of potassium intoxication. They did not develop diarrhoea, nor did they vomit, they just became weak and died. Likewise, we have seen a patient with marked limitation in tolerance for potassium due to advanced pan nephritis become fatally intoxicated with potassium as a result of drinking fruit juices.

Adolph: The study of tolerances is a very important aspect of the general physiology of regulatory processes. Dr Talbot, you estimated tolerances in terms of single constituents, but in some of the situations you described, such as the intravenous administrations, you were concerned with several constituents at a time. Now when there is depletion or excess of more than one constituent at a time the picture is very different with respect to tolerance. For instance, there is a great difference between taking pure salt and taking an isotonic solution of salt. I recognize that this work is exploratory and that you are making your estimates in the simplest way possible when you consider one component at a time, but eventually I hope we shall have some estimates of tolerance to multiple components.

This consideration of components seems to me to extend also to your studies of composition, Prof. Wallace. If you went to your statisticians still more often, would you not get into the study of multiple correlations which would get us further than comparisons made two at a time?

Wallace: We have made a number of statistical multiple correlations. It is often difficult to know just what they mean, once certain correlations become evident. Our biggest problem has been to have any assurance as to the proper parameter to which to refer growth. Should the reference basis be body weight, fat-free weight, protein, ash, or water?

Adolph: What I want to bring out is that an organism probably has some way of measuring the bodily composition which is very much more complicated than saying, for instance, that magnesium is the fixed constituent around which all others revolve. I think that without a study of multiple correlations we will never be able to find whether there is a key fixity by which homeostasis is guided to a definite volume and concentration to which the organism always returns. I do not know whether any of our methods of representing homeostasis will be so similar to that of the organism that we can predict what it does to get back to its fixity.

I should also like to remark on Dr Talbot's choice of a key variable. No doubt he has great reservations about the use of this term. What he is trying to do, I gather, is to out-guess the organism as to what it is using as a measuring stick by which it will return to its original composition, or by which it will estimate what has to be done in order to defend itself against disturbances. When we think that an organism is restoring its potassium concentration, have we any assurance that that one restoration is a prime objective in the adjustments which are going on?

Talbot: We agree with you that most if not all of the variables under consideration are related to each other. For instance, it is known that body tolerance for potassium is impaired under conditions of zero sodium intake and that tolerance for sodium is abnormally limited under condi-

(4) Partly due to the interrelationships (2) and (3) kidney function is readily impaired by stress

Thus the high incidence of body fluid disturbances is partly due to the occurrence of disease and partly to relatively inefficient homeostatic defence mechanisms The latter is well

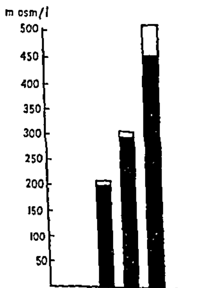


FIG 1 Lability of osmotic regulation in 10 day-old puppies.

Left column	salt and protein free diet
Central column	normal
Right column	concentrated milk

illustrated by the observation that diets such as milk evaporated to one-quarter of its original volume, or salt and protein free food bring about great changes in the tonicity of the body fluids (Csapó and Kerpel Fronius, 1933, Kerpel Fronius, 1933) After the first, the osmolarity of the blood plasma in puppies rose to 520 m osm /l, 457 m-osm being accounted for by 'hyperelectrolytaemia', after the second, the electrolytes decreased to 232 m osm /l (Fig 1) There

CLINICAL CONSEQUENCES OF THE WATER AND ELECTROLYTE METABOLISM PECULIAR TO INFANCY

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Disturbances in the volume and composition of the body fluids occur more frequently in infancy than at other ages Among the reasons for this are

(1) The high incidence of diarrhoea, malnutrition, and certain congenital defects

Diarrhoea is still one of the paediatrician's major concerns, one of its main causes being colon bacilli, pathogenic only for this age group

Owing to their high caloric and protein requirements infants easily succumb to malnutrition, which progresses rapidly The resulting expansion of the volume of their extra cellular body fluids, sometimes accompanied by asympto matic hyponatraemia, is a common disturbance of homeostasis in some countries

Congenital defects of the oesophagus, the pylorus, the renal tubules, the adrenals, and the central nervous system may also cause serious disturbances in the body fluids, their discussion is beyond the scope of this paper

(2) *Circulation, metabolism and renal excretion* are all maintained at high levels relative to the volume of the body fluids

(3) When growth is arrested by disturbances which diminish the utilization of food, a fraction of the intake normally retained is rejected, thus raising the solute load on the kidneys

* In the absence of Prof Kerpel Fronius his paper was read for him by Dr Winifred Young

(4) Partly due to the interrelationships (2) and (3) kidney function is readily impaired by stress

Thus the high incidence of body fluid disturbances is partly due to the occurrence of disease and partly to relatively inefficient homeostatic defence mechanisms The latter is well

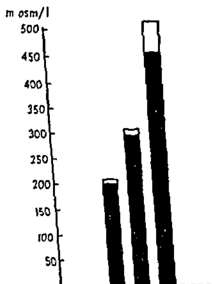


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Left column	salt and protein free diet
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illustrated by the observation that diets such as milk evaporated to one quarter of its original volume, or salt- and protein-free food, bring about great changes in the tonicity of the body fluids (Csapó and Kerpel Fronius, 1933, Kerpel Fronius, 1933) After the first, the osmolarity of the blood plasma in puppies rose to 526 m osm /l, 457 m-osm being accounted for by "hyperelectrolytaemia", after the second, [illegible] decreased to 232 m osm /l (Fig 1) There

was a water loss of over 20 per cent of body weight in the first case, while in the second an increase in the water content of all organs was observed Such gross disturbances of homeostasis may partly be due to the fact that although the extracellular body fluids occupy a relatively high percentage

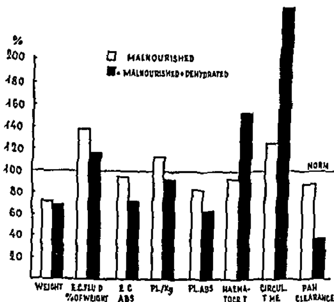


Fig 2 Extracellular fluids circulation and PAH clearance in the dehydration of a malnourished infant
Values are represented as percentages of those found in nd

White column before diarrhoea
Black column after diarrhoea
E C — extracellular PI — Plasma

of the body weight, the water reserves in infants are low in relation to the functions they may be called upon to perform

In order to reconcile this apparent contradiction, it is helpful to consider the relationship of body fluid reserves to circulation and kidney function in malnourished infants Malnutrition does not affect all systems of the body equally, fat and muscle sustaining greater losses than the extracellular

fluid compartment Hence the size of the latter appears to increase with the progress of malnutrition (Kerpel Fronius and Kovach, 1948, McCance, 1951, Keys *et al*, 1950) Haemodynamically, however, it is not the amount relative to body weight but the absolute amount of extracellular fluid which is of importance Fig 2 illustrates a striking example of a case studied in comparison with well nourished infants of the same length, first in a state of malnutrition and later after dehydration due to diarrhoea had supervened In the malnourished infant the volume of the extracellular fluid showed a percentage increase before and even after diarrhoea However, the ' absolute amounts", i e the fluid volumes calculated as percentages of those in normally nourished infants of the same length, were decreased Since the haematocrit readings were high the circulation time prolonged, and the renal clearances low, high water reserves calculated as a percentage of the body weight were clearly insufficient to maintain circulation and kidney function The absolute volume of the water reserves and not just the amounts proportional to the body weight must be maintained in order to conserve a normal circulation and good renal function

Let us now consider the normal infant When compared with the adult, his extracellular water reserves—although high in terms of percentage of body weight—are strikingly low in relation to other physiological needs, namely oxygen consumption insensible perspiration and cardiac output (Fig 3)

Thus when compared on the basis of *body surface*, the infant appears to have the same oxygen consumption and cardiac output as the adult, but his systolic output (stroke volume) and plasma volumes are only half those of the adult, in order to achieve the requisite cardiac output with a relatively low plasma volume, the pulse rate is double that of the adult His inulin and *p* aminohippuric acid (PAH) clearance values are low in comparison with those of the adult and also in relation to his own cardiac output and metabolism All his fluid compartments are strikingly low in proportion to metabolism, insensible perspiration and cardiac output

Alternatively, on the basis of *body weight*, the infant's metabolism, dermal loss of water and cardiac output appear to be very high in relation to his total body water and plasma volume, which occupy approximately the same space as in the

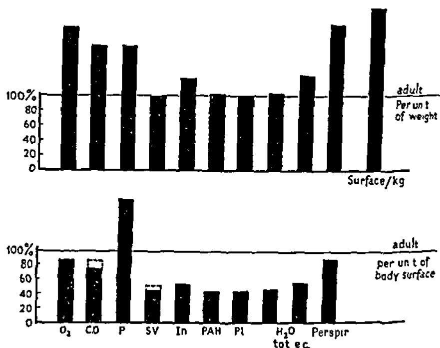


FIG 3 Haemodynamics fluid spaces and renal function of the infant a percentages of values for the adult

... present mean values for five infants aged 4 months with bod... as of 0 30 m² Th... unit of body weight ... show

C O — cardiac output P — pulse rate, S V — systolic volume, In — inulin Pl — plasma e c — extracellular

adult This relationship holds true also for the extracellular fluid volume, although this is higher than in the adult Renal clearances are proportional to fluid volumes and therefore low in relation to circulatory and metabolic rates

Despite the marked differences between adults and infants in some of the physiological constants which have been mentioned these functions are certainly nicely adjusted to each other even in the infant, and his defence mechanisms are fully capable of meeting the normal demands upon them When put under stress however, the fragility of the whole system which maintains body fluid homeostasis is exposed

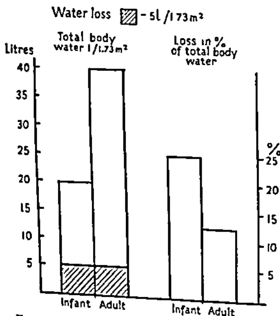


FIG 4 Significance of "equal" losses when expressed per unit of body surface

Under pathological conditions the consequences of the peculiar interrelationship of these functions are as follows

(a) Water or salt loads calculated according to surface area will in relation to total body water content, be double the values of the adult The same holds true for loss of water, equal losses per unit of surface area being twice as high in the infant in proportion to the body water (Fig 4)

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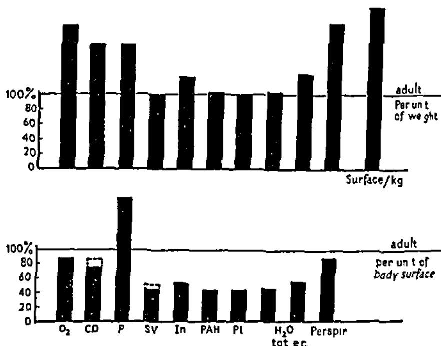


FIG 3 Haemodynamics fluid spaces and renal function of the infant as percentages of values for the adult

— ... mean val es for five infants aged 4 months, with *body* ... m² The ... ly weight, ... nt, shows ... ercentage differences between ad...

C O — cardiac output P — pulse rate, S V — systolic volume, In — inulin, Pl — plasma, e c — extracellular

adult This relationship holds true also for the extracellular fluid volume, although this is higher than in the adult Renal clearances are proportional to fluid volumes and therefore low in relation to circulatory and metabolic rates

may be threatened either by high urine volumes or, in the case of renal inadequacy, by uraemia

In summary, the mechanisms defending body fluid equilibrium in the infant are more easily broken down owing to the water reserves being low in relation to the high metabolic rate and "strained" circulation In circumstances of shortage this small water pool is quickly exhausted, and it is also easily flooded by loads which, in terms of body surface, are equal to those for adults By decreasing the small plasma pool rapidly, water losses lead to slowing down of circulation Owing to the rapidly decreasing renal clearances, as well as the high metabolic rate producing solutes at great speed, the relatively small water pool cannot then keep up its constancy. Deterioration is accelerated by arrested growth.

In conclusion a particular type of dehydration in which the infant seems to be in a somewhat less difficult position than the adult may be mentioned In infantile pyloric stenosis, a condition in which starvation and dehydration develop together, a sharp decrease of about 50 per cent in oxygen consumption has been observed by Varga (1957). We have found that this diminution in oxygen requirements protects against stagnating anoxia brought about by the slowing down of circulation due to dehydration (Kerpel F... ...st probably ... thus delay ... Since the metabolic rate decreases less in the semi starved adult (Keys *et al*, 1950), the infant may possibly be more resistant to dehydration when he is already suffering from starvation than an adult under similar circumstances

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(b) Water deprivation quickly exhausts the water reserves which are low in relation to metabolism and, consequently, to obligatory urine volume and dermal loss of water

(c) Because of the high cardiac output required for metabolic processes, and the low reserves of water to guarantee its maintenance, circulation is endangered by even smaller water deficits, the more so since water losses occur rapidly It will be remembered that the small plasma volume of the infant relative to the cardiac output is compensated for by a high pulse rate to ensure adequate circulation

(d) The vulnerability of the circulation facilitates a rapid decrease in renal clearances, which even in the healthy infant are low in relation to his high metabolic rate Obviously, the *infant's rather poor renal blood flow is adjusted to, and only* maintained by a relatively high cardiac output The renal fraction has been calculated to be 10 per cent of the total output of the heart in infants whereas it is 20 per cent in adults

As pointed out by McCance and Widdowson (1957) stagnation of growth plays a rôle in the easily disturbed equilibrium In a growing animal a certain amount of the food goes to the building of its tissues If growth is arrested, an additional solute load formed by this fraction of the intake presents itself for excretion by the kidneys This will result either in a higher urine volume, or, if the kidneys are incompetent, in hyperelectrolytaemia and azotaemia McCance and Widdowson (1957) have shown that these effects are striking in fast growing animals and may under certain circumstances be of *importance to the human infant* On the basis of some of the data compiled by the American Academy of Pediatrics (1957) an *estimate has been made of the effect of arrested growth on* solute load and renal water expenditure Solute load may be expected to rise 18 per cent *in the infant who is fed on cow's* milk, and 57 per cent in the breastfed child causing a considerable increase in urine volume When *at the same time* extrarenal water expenditure is increased by high environmental temperature, or diarrhoeal losses, the *water balance*

... with a very high pulse rate and low cardiac output there must be ...

Young The great value of this paper is in explaining why the baby is more ...

Heller We are always talking about the large body water content or the high ...

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DISCUSSION

Davson Has the subject of size *per se* been considered as oppose immaturity? The pulse rate of the baby was mentioned as being fa than that of the adult and the reasons for it were based on the *maturity of the organism, whereas one finds that small adult ani* have very fast pulse rates The rabbit pulse, for instance, is well into hundreds and the mouse pulse is even faster

Young I do not think it has been suggested that the pulse rate is h because of immaturity it is high because of the high metabolic rat relation to the other constants, and in order to keep up the cardiac out

Adolph The effect of body size on functions such as pulse rate respiration rate varies considerably in any one species Among vari

that none of the body size rules apply uncomplicatedly during *infan* There are other factors, and perhaps the factor of metabolic peculiari is one of them

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Young When this infant became dehydrated he still had a relativ high volume of extracellular fluid as a percentage of body weight, b

THE EFFECT OF HORMONES OF THE PITUITARY AND ADRENAL GLANDS ON THE ELIMINATION OF SODIUM, POTASSIUM AND A WATER LOAD IN INFANT RATS DURING THE WEANING PERIOD

Jiří Křeček, Helena Dlouhá, Jiří Jelinek, Jarmila Křečkova and Zděnek Vacek

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Homeostatic mechanisms in infant animals differ from those in adults of the same species Mechanisms regulating the metabolism of water and electrolytes change immediately after birth, during the period the eyes open, at the time of weaning in connexion with sexual maturation and perhaps also at other stages of postnatal development In the present paper we should like to draw attention to the time of weaning which seems to us to be one of the important stages in the development of the regulation of water and electrolyte metabolism

The preweaning period in rats is relatively long Up to the 14th day of life infant rats cannot survive without the mother rat They are usually weaned at the end of the third week but according to breeders natural weaning occurs only at the end of the fourth week This agrees with the development of thermoregulation, for infant rats can survive very low environmental temperatures without the mother only at the end of the fourth week (Čapek *et al*, 1956)

Up to the 14th or 18th day infant rats live on breast milk only This is the only source of water and electrolytes, if we disregard the urine of litter mates that is sometimes sucked by the infant animals From that time onward infant rats in addition to breast milk also actively feed on solid food and

nearer its adult composition in foetal life than the skeletal muscle I think a great deal of this change is in the skeletal muscle and not in connective tissue.

Fourman: Then is there a difference in the mode of growth of skeletal muscle on the one hand, and liver and heart on the other? Does skeletal

Fourman. If the extracellular fluid is considered as a film over the cells, 'lular fluid

test that, because cell size data based on nucleic acid determinations are available for many different ages in a number of species, and equally, extracellular fluid determinations are available in the same tissues.

ment

Fourman Dr Shock, is the water content in the muscle larger in old people than in the young ones, since muscles do atrophy in old age? We have had that answered indirectly in Dr Olesen's paper, but are there any direct analyses?

Shock I cannot answer for the human, but we have some data on the electrolyte and water composition of rat muscle tissue We found that

decreased. The potassium, phosphorus, and nitrogen contents all went down, but the chloride and sodium contents went up. The ratio of potassium to nitrogen and of phosphorus to nitrogen remained constant Our interpretation of this was in the light of our beliefs about the reduction in active protoplasm in old age It is as if a certain mass of protoplasm had disappeared and been replaced by extracellular compounds with the appropriate amount of sodium and chloride to make up the total water composition.

Fourman As I said, it is not a replacement, but—to borrow Dr. Davson's expression—a geometrical necessity to keep a film of water around the cells.

Kennedy: But you would need to know whether the atrophy was due

f the kidneys to eliminate an administered water load hanges and its ability to concentrate increases According o Falk (1955), however, infant rats older than three days lready react to vasopressin by cessation of diuresis and an ncreased excretion of chloride As both authors use different nethods it seemed useful to study this problem first, using several methods, and also to study the effect of vasopressin on the elimination of sodium and potassium Opinions on the natriuretic effect of vasopressin also differ and we believe that this is due to different methodological approaches Schaumann (1949) and Heller and Stephenson (1950) observed that vasopressin decreases the excretion of sodium in adult rats, while Sawyer (1952) observed an increased elimination of this electrolyte The former authors administered the hormone at the same time as the water load Sawyer first slightly prehydrated his animals and then gave them the hormone and the water load According to Heller (1952) the ability of the rat kidney to eliminate a water load changes at the time of weaning We therefore always used rats with a water load

Infant rats were weaned on the 15th 16th day after birth and the whole litter left in one cage They received a standard synthetic diet without sodium chloride They were allowed to choose between water and a 3 per cent sodium chloride solution As we expected changes in the mechanisms studied to occur at the end of the fourth week, infant animals aged 23 and 33 days were used Loads of warm distilled water were administered via a stomach tube in amounts of 4 5 ml /100 g body weight Subcutaneously the animals received saline (0 5 ml /100 g body weight) in which the substances studied were dissolved The elimination of a water load was studied for three hours after its administration or, in the case of vasopressin, for three hours from the first micturition Urine was collected at hourly intervals The amount of urine, together with the concentration of sodium and potassium, was determined by use of a flame photometer.

Adult rats rapidly excrete urine with a low content of

drink water Gradually the mechanisms for compensation of thirst and hunger separate At the end of the fourth week infant animals cease to feed on breast milk and take in food that is normal for adult animals

We studied the active intake of water, electrolyte solutions and milk in infant rats using the method of free choice as known especially from the work of Richter (1936), Young (1949), and Young and Chaplin (1949) We observed that in infant rats weaned at the beginning of the third week of postnatal life there is a significant change in the regulation of water, electrolyte and milk intake at the end of the fourth week The regulation of sodium intake in relation to water intake, especially, changes According to Richter (1936) appetite for individual components of the diet is an important homeostatic mechanism and is determined by the needs of the organism

In order to be able to offer a physiological explanation for changes in the regulation of sodium intake it is necessary to throw light on the relation between mechanisms of self selection and other components of water and electrolyte metabolism that can be studied better and more objectively

The adrenals and the posterior lobe of the pituitary are of special significance for the regulation of water and electrolyte metabolism For this reason we have studied the effects of hormones from these two glands Up to the present nothing is known of a change in function of the adrenals or in the effect of their hormones at the end of the fourth week of life in the rat Indirectly one might expect such a change from the fact that the regulation of sodium intake depends on the function of the adrenals (Richter, 1936) There is also no difference in the size of the glands in males or in females during the fourth week

More is known about changes in the rôle played by the posterior lobe of the pituitary during this period Heller (1952) showed that up to the end of the fourth week of life the rat kidney does not react to vasopressin during a water load in the same way as that of the adult In addition the ability

Older animals, however, excrete nearly half the water ad ministered and thus excrete body water via the kidneys Differences in sodium excretion are also apparent Thirty three day-old animals excrete three times as much body sodium as younger rats The difference between both age groups studied disappears completely, or becomes much smaller, if 2 5 ml water/100 g body weight is put into their stomachs two and a half hours before the actual water load In that case more urine is excreted by the younger animals and losses are reduced in the older age group Sodium losses are also decreased in the older age group to the same level as in 23 day-old animals No significant changes in potassium excretion were observed

Differences between the two age groups are thus not con stant For this reason we assume that the difference is not due only to changes in renal function but that regulatory mechan

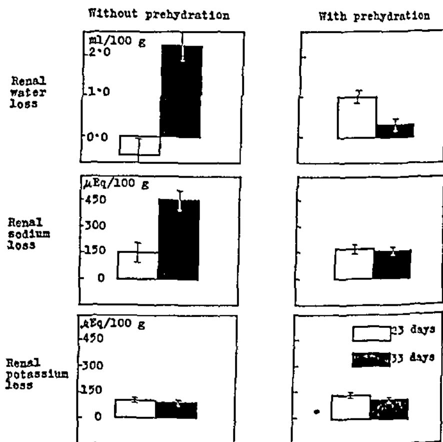
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the water load was studied according to the method of Falk (1955) In addition the effect on total water loss three hours after the first micturition was studied This procedure was similar to that of Heller (1952) who determined total renal excretion of a water load 145 minutes after administration of the hormone and the water load

After 10 or 25 m u vasopressin/100 g body weight, no significant differences between the two age groups could be observed during water diuresis This is in agreement with Falk (1955) Yet 23-day-old animals react differently to vasopressin than 33-day-old rats This difference can be seen in Table I After a single water load vasopressin (the table shows the results with 25 m u /100 g body weight) increases renal water losses in the younger animals, while in the older group total renal water losses are reduced The sodium loss in older animals treated with vasopressin becomes greater after prehydration only In younger animals the elimination of potassium is significantly greater than in the

sodium and potassium after administration of a water load Males excrete a water load less well than females

In our experiments the excretion of a water load was the same in infant rats as in the experiments of Heller (1952)



There are no sex differences There are, however, considerable differences between infant animals aged 23 and 33 days These can be seen in Fig 1. Twenty-three day old animals do not eliminate the total water load within three hours

Older animals however, excrete nearly half the water ad ministered and thus excrete body water via the kidneys Differences in sodium excretion are also apparent Thirty three day old animals excrete three times as much body sodium as younger rats The difference between both age groups studied disappears completely, or becomes much smaller, if 2 5 ml water/100 g body weight is put into their stomachs two and a half hours before the actual water load In that case more urine is excreted by the younger animals and losses are reduced in the older age group Sodium losses are also decreased in the older age group to the same level as in 23-day-old animals No significant changes in potassium excretion were observed

Differences between the two age groups are thus not con stant For this reason we assume that the difference is not due only to changes in renal function but that regulatory mechanisms are also concerned

The effect of vasopressin was studied in animals receiving one water load and in prehydrated rats The elimination of the water load was studied according to the method of Falk (1955) In addition the effect on total water loss three hours after the first micturition was studied This procedure was similar to that of Heller (1952) who determined total renal excretion of a water load 145 minutes after administration of the hormone and the water load

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Table I

THE EFFECT OF VASOPRESSIN ON SODIUM AND POTASSIUM EXCRETION AND WATER LOSS AFTER WATER LOAD IN PREHYDRATED AND UNPREHYDRATED RATS OF DIFFERENT AGES

	Age of animals in days	Sodium excretion		Potassium excretion		Renal water loss	
		No of animals	µ equiv / 100 g body wt /3 hr	No of animals	µ equiv / 100 g body wt /3 hr	No of animals	ml /100 g body wt /3 hr
Water load 4 5 ml /100 g body wt H_2O via stomach tube + 0 5 ml./100 g body wt saline s c without prehydration	23	7	147 ± 50 4	8	99 ± 9 7	8	−0 5 ± 0 4
	33	8	450 ± 45 5	7	85 ± 13 7	8	2 3 ± 0 38
Water load 4 5 ml /100 g body wt H_2O via stomach tube + 25 m u / 100 g body wt vasopressin in 0 5 ml /100 g body wt saline s c without prehydration	23	7	187 ± 28 3	5	123 ± 62 5	7	0 9 ± 0 36
	33	8	350 ± 30 0	7	113 ± 18 1	7	1 4 ± 0 18
Water load 4 5 ml /100 g body wt H_2O via stomach tube + 0 5 ml / 100 g body wt saline s c with prehydration	23	15	168 ± 24 3	16	135 ± 13 0	15	1 0 ± 0 24
	33	14	154 ± 21 3	14	109 ± 11 4	16	0 3 ± 0 215
Water load 4 5 ml /100 g body wt H_2O via stomach tube + 25 m u / 100 g body wt vasopressin in 0 5 ml /100 g body wt saline s c with prehydration	23	16	245 ± 36 2	10	173 ± 10 3	16	1 4 ± 0 32
	33	13	255 ± 29 0	13	123 ± 13 0	16	−0 1 ± 0 41

33 day old rats Thus vasopressin has a different effect in 23 day old than in 33 day old animals Evidently there is a

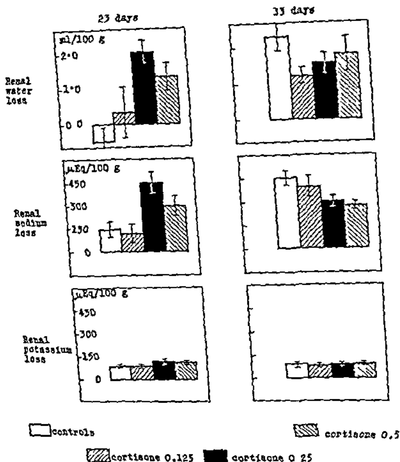


FIG 2 The effect of cortisone administered for six days in different doses on renal loss of water sodium and potassium during the first three hours after administration of a water load (4 5 ml /100 g body wt) to young rats aged 23 and 33 days

change in the reactivity of the kidneys to this hormone at that period This might be due to functional differences in

kidney parenchyma or to the fact that from the end of the fourth week a regulatory factor is present which can be influenced by loading the organism with water It therefore seemed all the more interesting to us to find out whether the *function of the adrenals* changes at the time of weaning

After adrenalectomy the ability to eliminate a water load is strongly reduced in infant rats It is difficult therefore to use this method for solving the problem A less direct way was chosen — a study of the effect of substances that act in a similar way to the main corticoids Cortisone or cortexone was administered for six days in various doses to 18–23 and 28–33 day old animals Then a water load was given It appeared that the effect of these substances also depends on the age of the rats

The effect of cortisone is shown in Fig 2 The elimination of a water load, sodium and potassium was determined in rats that received 0 125, 0 25 or 0 5 mg cortisone/100 g body weight The hormone has opposite effects in the younger and in the older age groups In 23 day old animals it increases the excretion of water (as it does in the 8 day old rats of Falk, 1955) and sodium, while in the 33 day old rats it decreases both After a dose of 0 25 mg /100 g body weight, renal water and sodium losses in the younger animals reach
... the older control animals It
... of cortisone compensates for
... r animals but present in the
older rats This, however, is not borne out by the way in which a water load is eliminated by the younger rats after cortisone Fig 3 shows changes in the concentration of sodium in the urine during the course of water diuresis in normal animals and after cortisone (0 25 mg /100 g) In the control 33 day old animals the concentration rises as the intensity of water diuresis falls In the younger group there is no such relationship and the concentration is not lowest during the highest diuresis If cortisone were only a substituting substance the course of the curves of sodium concentration ought to be the same in 23 day old rats receiving cortisone

and 33-day old controls As this is not the case and as in the younger animals increased natriuresis is mainly due to increased concentration at the time of maximum water

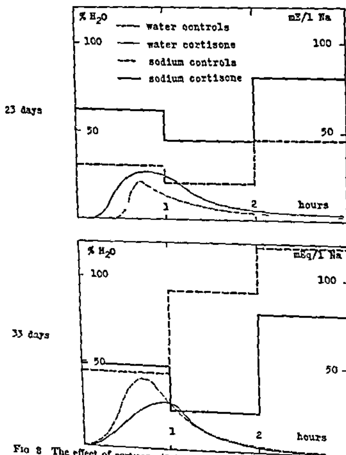
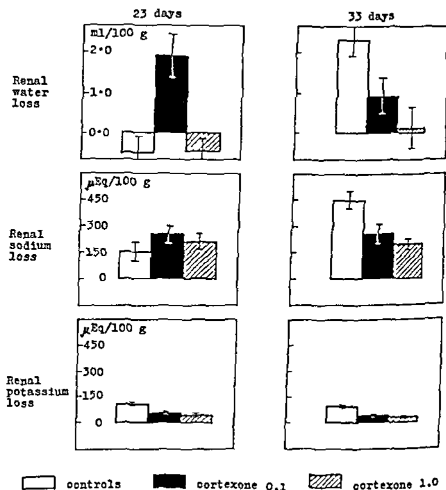


FIG 8 The effect of cortisone (0 25 mg /100 g body wt./day) on the course of the excretion of a water load and the concentration of sodium in the excreted urine in infant rats aged 23 and 33 days

diuresis, relations are evidently more complex This is also borne out by the fact that the effect of cortisone in the younger group is variably dependent on the dose used

This is even more evident in the case of cortexone. This was administered by the same route as the former substance



[illegible]

but in doses of 0·1 and 1 mg./100 g. body weight. Results are shown in Fig. 4. Lower doses of cortexone had an effect similar to cortisone, quantitatively different in younger and

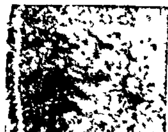
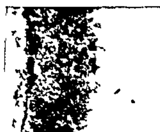
	23 days	33 days
Controls		
Cortisone		
ACTH		
Cortisone + ACTH		

Fig. 5. The effect of cortisone (0.2 mg./100 g. body wt./day) and ACTH ([illegible] i.u./animal/day) administered for six days on the size of the adrenal cortex in young rats aged 23 and 33 days. Stained with Sudan Black.

older animals In younger animals it increased renal losses, which thus nearly reached the levels of the older controls In 33 day old animals water losses decreased after cortexone The higher dose, however, had no effect on renal losses of water in 23 day old animals, whereas in 33 day old rats it further decreased renal losses These doses, however, are probably toxic Sodium losses were never significantly altered by either dose of cortexone in the younger group In 33 day old animals they changed in direct proportion to the dose used In both age groups cortexone decreases renal potassium losses significantly

Thus corticoids have a different effect on the elimination of water and electrolytes after a water load in infant rats that have not yet reached the age at which they are normally weaned, than they have in older animals The opposite effects in 23 day old animals, depending on the dose used, indicate that these hormones cause changes that mutually interfere with each other

We attempted to determine whether in addition to the pharmacodynamic effect of these hormones there is also an effect on the regulation of adrenal activity

The weight of the adrenals of animals receiving cortisone or cortexone, as indicated above, dropped to about the same extent in both 23 and 33 day old animals Simultaneous administration of ACTH in amounts usually sufficient to maintain adrenal weights of hypophysectomized animals (0 2 i u per animal) prevents adrenal atrophy in both groups This reaction is less obvious on histological studies Fig 5 shows microphotographs of the adrenal cortices of 23 and 33 day old [illegible]

1 [illegible] and ACTH) Preparations were stained with Sudan Black so that both the width of the cortex and the sudanophil layers can be seen After ACTH there are no obvious changes in the width of the cortex and the sudanophil layer After cortisone and cortisone plus ACTH differences are evident This is even more apparent

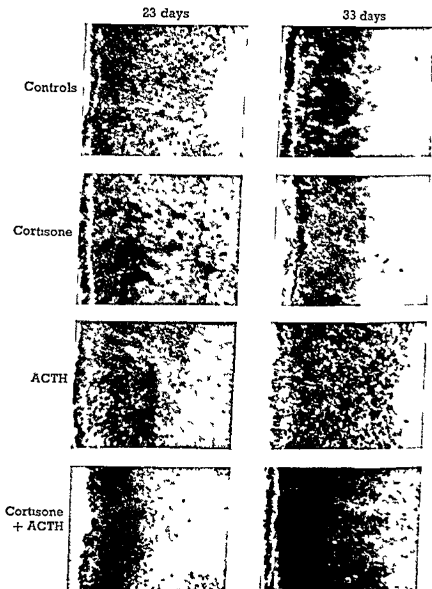


FIG 5 The effect of cortisone (0 25 mg /100 g body wt /day) and ACTH (0 2 i u /animal/day) administered for six days on the size of the adrenal cortex in young rats aged 23 and 33 days Stained with Sudan Black

of the sudanophil layer increases in both age groups but in the younger group the size of the whole cortex is smaller It is difficult to interpret these changes It is certain, however, that according to morphological criteria the adrenals of the 23-day old animal react differently from those of the animal aged 33 days This would indicate that changes in the reactivity of infant rats to a water load at the end of the natural period of weaning and to corticoids are also conditioned by a different reactivity of the adrenals and the adrenopituitary system

This hypothesis is further supported by results from experiments in which the effect of ACTH and a combination of ACTH and cortisone (0 25 mg /100 g) was studied on the elimination of water, sodium and potassium after a water load Results are shown in Fig 7 As has already been shown, cortisone prevents retention of a water load in 23 day old animals and considerably increases renal water losses ACTH is without effect After simultaneous administration of ACTH and cortisone, water losses decrease in comparison to losses after cortisone only In 33 day old rats results are less evident because of the large scatter ACTH itself causes an increase in sodium excretion in 23 day old animals but in combination with cortisone it is without effect on sodium elimination and thus removes the latter's natriuretic effect This effect is probably due to the lower renal water losses In 33 day old animals ACTH decreases sodium losses just as do cortisone and cortisone combined with ACTH The same holds good for ACTH when combined with cortexone ACTH prevents atrophy of the adrenals after cortisone in infant rats aged 23 days and also prevents the effect of cortisone on sodium and water elimination This is not the case in older animals This is in agreement with the histological picture and with the differences between 23 and 33 day old animals

We have thus been able to show that there is a time correlation between changes in homeostatic mechanisms regulating the intake of water and electrolytes appearing in infant rats at the time of natural weaning, and adrenal

in Fig 6, which shows the results of micrometric measurements of the width of the cortex and the sudanophil layer as obtained from serial sections of the adrenals Four adrenals from each group were measured One hundred sections from each gland were used and measurements were taken from

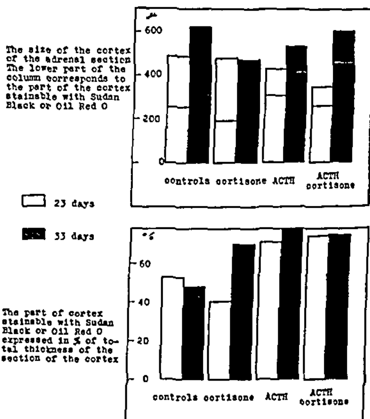


Fig 6 See Fig 5

several sites of those sections Differences are largest after cortisone In 23 day old animals the sudanophil layer decreases in size while the total width of the cortex remains unchanged In 33 day old animals the width of the cortex decreases and thus the relative width of the sudanophil layer is increased After ACTH and cortisone the proportion

This is probably conditioned by the presence of a regulating mechanism which after previous loading with water increases the reabsorption of sodium At the end of the preweaning period there is a considerable change in the effect of cortisone and cortexone on elimination of water and sodium after a water load Even 33 day old animals, however, do not react quantitatively in the same way as adult animals This is evidently due to the fact that only after the 33rd day does the male adrenal begin to differ from that of the female. It may be assumed from the results presented here that the reactivity of the adrenals changes at the time of weaning That change can be in relation to the change in homeostatic mechanisms regulating the intake of water and sodium which occurs at the time of weaning

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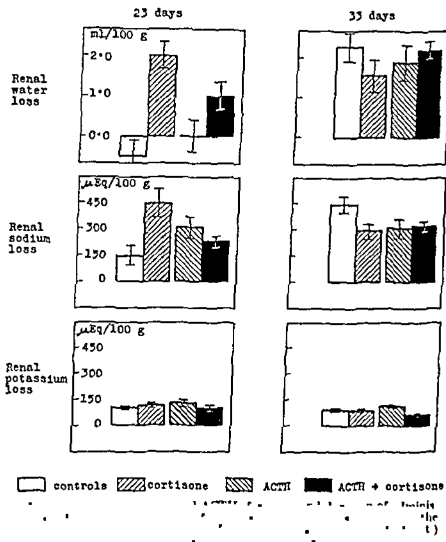
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[Discussion of this paper was postponed until after the paper by Dr Desaulles —EDS]

pituitary mechanisms regulating the metabolism of water and electrolytes At the end of the fourth week of life the effect



of vasopressin on elimination of a water load changes This is in agreement with Heller (1952) In addition, at this time vasopressin begins to have an effect on sodium elimination

own adrenals, as well as to avoid strain bound differences in sensitivity

To test the action of steroids on urinary and electrolyte excretion, we have used the method described in detail by Desaulles and Meier (1956), the only difference being that, instead of collect ng ... ine from the fifth ...

first to the third hour, from the second to the fourth hour, and from the seventh to the ninth hour following treatment, this procedure enabling us to follow closely the excretion of urine and of electrolytes Both male and female animals were used, the age groups being

(a) animals about five weeks old and about 50 g in weight,
(b) animals about 15 weeks old and about 150–180 g in weight,
(c) animals about one year old and exceeding 300 g, in weight

All animals used in these experiments were kept isolated in metal ...
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The steroids chosen, aldosterone and cortisol, are known to be secreted by the rat adrenals (Bush, 1953, Singer, 1957) Cortisol was used as free alcohol, aldosterone was used as DL aldosterone acetate, the activity of which is just one half of D aldosterone (Schmidlin *et al*, 1955, 1957) All substances were dissolved in sesame oil and injected intramuscularly

The doses used in these experiments were chosen from previous experiments (Desaulles and Meier, 1954, Desaulles, 1958) and lay within a dose range corresponding to sub maximal effects For aldosterone acetate 0 01 mg /kg was given, and for cortisol 5 mg /kg

As the excretion of urine and urinary electrolytes differs

DIFFERENCES IN THE PATTERN OF ELECTROLYTE AND WATER EXCRETION IN YOUNG AND OLD RATS OF BOTH SEXES IN RESPONSE TO ADRENAL STEROIDS

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It is a known fact that, with advancing age, the cell mass and, correspondingly, the cell water content of the animal decrease. This, together with a constant or increasing extracellular water content, appears to be one of the true signs of ageing (McCance and Widdowson, 1951, Olbrich and Woodford-Williams, 1956).

Although the adrenals, and more especially the adrenal steroids, play an important part in the maintenance of the water and electrolyte balance, only comparatively little is known about the influence of age on the activity of the adrenals or on the sensitivity of the organism to adrenal steroids in animals. We were therefore prompted to study in rats of different ages the pattern of urine and urinary electrolyte excretion after treatment with two genuine adrenal steroids, aldosterone and cortisol, following a load of physiological saline solution amounting to 20 ml per kg.

In view of the very complex interrelationship existing between pituitary, gonads, and adrenals during the development of the animal from birth to maturity and old age, we have also studied rats of both sexes. These animals were chosen in three different groups, ranging in age from (a) five weeks to (b) fifteen weeks to (c) one year and more.

Methods

All experiments were performed on adrenalectomized rats of the same breed, in order to avoid interference between the steroids injected and the steroid output of the animal's

Aldosterone prevents sodium excretion in a very marked manner in about the same intensity and for about the same duration (five to seven hours) in all age groups (Fig 2)

The only difference to be noted is that in old animals the onset of the sodium retaining effect of the steroid is retarded,

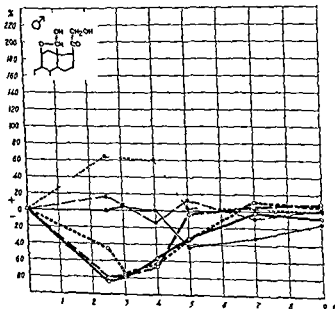


Fig 2 Urinary sodium and potassium excretion of adrenalectomized male rats of different age groups treated with aldosterone (0 010 mg /kg)

Thick line sodium excretion
Thin line potassium excretion.
Other figures as for Fig 1

the maximal effect falling in the collecting period of the third hour instead of in the preceding period

The effects of aldosterone on potassium excretion depend upon the age groups in question

In young animals aldosterone does not affect potassium excretion until the fourth collecting hour From the fifth

in amount in animals of differing age and weight, the results are expressed as percentages of the values of control animals for urinary excretion in ml, and for sodium and potassium excretion in m mole The differences between the sodium/potassium ratios of treated and control animals are, on the other hand, expressed in absolute values

Results

Effect of aldosterone

In the *male rat*, aldosterone produces a marked inhibition of urinary output that is most pronounced in young animals

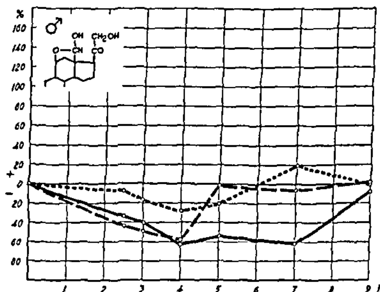


FIG 1 Urinary excretion of adrenalectomized male rats of different age groups treated with aldosterone (0 010 mg /kg)

Abscissa Duration of experiment (hours) collecting period 2 hours

Ordinate Urinary excretion as a percentage of the values of control animals

Continuous line 5 week-old rats
Interrupted line 15 week old rats
Dotted line one year and more old rats

and tends to diminish—at first in duration and then in intensity—with increasing age (Fig 1)

Aldosterone prevents sodium excretion in a very marked manner in about the same intensity and for about the same duration (five to seven hours) in all age groups (Fig. 2).

The only difference to be noted is that in old animals the onset of the sodium retaining effect of the steroid is retarded,

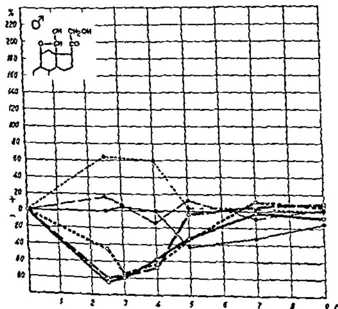


Fig 2 Urinary sodium and potassium excretion of adrenalectomized male rats of different age groups treated with aldosterone (0 010 mg/kg)
Thick line sodium excretion
Thin line. potassium excretion
Other figures as for Fig 1

the maximal effect falling in the collecting period of the third hour, instead of in the preceding period

The effects of aldosterone on potassium excretion depend upon the age groups in question.

In young animals, aldosterone does not affect potassium excretion until the fourth collecting hour. From the fifth

hour onward it induces a clear cut reduction in potassium excretion, which reverts to normal in the ninth hour On animals of the adult group aldosterone has practically no effect at all In *old rats, however, aldosterone markedly* enhances potassium excretion

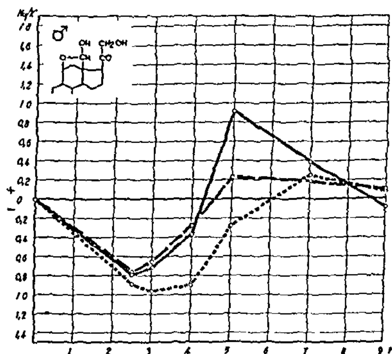


Fig 3 Urinary sodium/potassium ratio of adrenalectomized male rats of different age groups treated with aldosterone
Ordinate Difference between sodium/potassium ratio of experimental animals and controls
Other figures as for Fig 1

If we consider the sodium/potassium ratio, we observe that aldosterone reduces it markedly during the first hours of the experiment in all groups (Fig 3), its maximum occurring in the first collecting period *for young and adult groups, and* showing a certain delay (three hours) and greater intensity (−0 95 against −0 75 to −0 80) in the old age group From the fourth hour onward there is an increase in the ratio for

young animals (due to potassium retention), whereas adult and old animals return to a range within control values, the adult group reacting more readily than the old animals

In the *female rat*, aldosterone also reduces the urinary output, but to a somewhat smaller extent than in males (−40 per cent on the average, against about −60 per cent in males) (Fig 4) As in males, young animals tend to respond more



Fig 4 Urinary excretion of adrenalectomized female rats of different age groups treated with aldosterone (0 010 mg /kg)
Figures as for Fig 1

markedly although there is a certain delay in the onset of the effect In contrast to males, with increasing age a short period of urinary retention is followed by a strong diuretic response

On sodium excretion aldosterone exerts a very pronounced inhibiting effect of about the same relative intensity as in males in all age groups (Fig 5) In contrast to that in males, this effect is followed by a period of sodium excretion, most marked in old animals (+80 per cent), the values *returning* towards the norm in the ninth hour

On potassium excretion the enhancing effects of aldosterone are more marked and begin at an earlier age than in males, old animals showing the most pronounced effect

On the sodium/potassium ratio the effects are much more marked than in the case of males (Fig 6) Young animals respond with a reduction that is marked (−0 90), but of slow

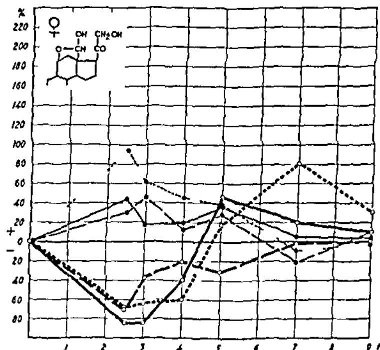


Fig 5 Urinary sodium and potassium excretion of adrenalectomized female rats of different age groups treated with aldosterone Figures as for Fig 2

onset (maximum in the fifth hour) the values returning to within control limits at the end of the experiment

In adult and old females the reduction in the sodium/potassium ratio is more intense (−1 29 and −1 10 respectively) and rapid in onset (maximum in the first collecting period) This effect lasts longest in old animals

The rapid lowering of the sodium/potassium ratio is

followed, in contrast to the situation for male animals, by a very pronounced and rapid rise (more in adult than in old animals) to high positive values (+0 95 and +1·28, respectively), this effect tending to return within control values in the ninth hour

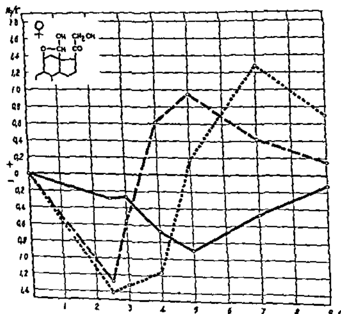


FIG 6

Effect of cortisol

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well developed, and ageing does not seem to modify it ... this effect is markedly (Fig 7)

On sodium, cortisol exerts initially a slight retaining effect that has already been reported (Dorfman, 1949, Johnson,

1954, Desaulles, 1958) and which is followed by enhanced sodium excretion (Fig 8) In males these effects tend to disappear with advancing age

On potassium, one observes the characteristic excretory response whose intensity is particularly high in young animals, its onset being somewhat more rapid in adult and old animals

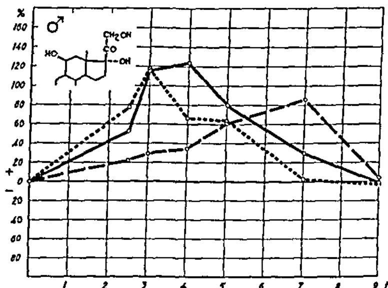


Fig 7 Urinary excretion of adrenalectomized male rats of different age groups treated with cortisol (5 mg /kg)
Abscissa Duration of experiment (hours) collecting period 2 hours
Ordinate Urinary excretion as a percentage of the values of control animals

Continuous line 5 week old rats
Interrupted line 15 week old rats
Dotted line one year and more old rats

The effect of cortisol on the sodium/potassium ratio is first to lower it moderately in males, and to raise it afterwards to high positive values (Fig 9) This effect, most marked in young animals, declines with increasing age

In *female rats*, cortisol has a stronger enhancing effect on urinary output than in males (Fig 10) With age, this effect increases and a certain latency of onset seems apparent.

On sodium, cortisol has similar retaining effects in females as in males and these disappear in old animals (Fig 11)

As regards the enhanced sodium excretion which appears later, females react differently from males Instead of dis

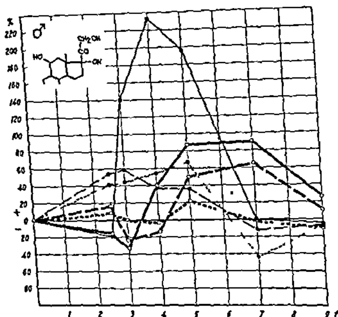


FIG 8 Urinary sodium and potassium excretion of adrenalectomized male rats of different age groups treated with cortisol (5 mg /kg)
Thick line sodium excretion
Thin line potassium excretion
Other figures as for Fig 1

appearing with increasing age, the response remains high and its onset is more rapid in ageing females, although in this experiment animals of the adult group do not respond clearly

The effect of cortisol on potassium excretion in females is similar to that observed in *males*, i e it is enhanced, the effects tending to decrease in intensity with age

FIG 9 Urinary sodium/potassium ratio of adrenalectomized male rats of different age groups treated with cortisol (5 mg /kg)

Ordinate Difference between sodium/potassium ratio of experimental animals and controls

Other figures as for Fig 1

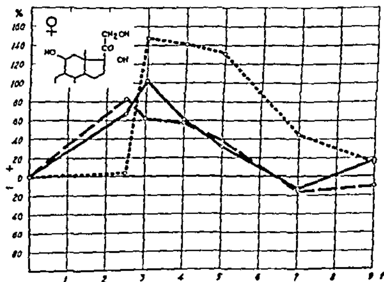


FIG 10 Urinary excretion of adrenalectomized female rats of different age groups treated with cortisol (5 mg /kg)

Fig 11 Urinary sodium and potassium excretion of adrenalectomized female rats of different age groups treated with cortisol (5 mg /kg) Figures as for Fig 2

On the sodium/potassium ratio, the effects are comparable to those obtained in males but are of greater intensity (more than twice the values observed in males) and of more rapid onset, and also tend to diminish rapidly with increasing age (Fig 12)

Discussion

From the experimental results presented, it follows that age modifies the sensitivity of adrenalectomized rats to the influence of the adrenal steroids investigated These modifications are qualitative as well as quantitative, the sex of the animals also playing an important rôle

Whereas in male rats increasing age tends to reduce to control values the inhibiting effects of aldosterone on urinary output, it tends in females to induce a marked secondary diuretic response The primary retention of sodium produced by aldosterone is of about the same order of magnitude in all animals, whether male or female, but ageing greatly increases the concomitant loss of potassium this effect being particularly clear in male and female rats of the old age group

In contrast, cortisol has an enhancing effect on diuresis which, especially in females tends to increase with advancing age, whereas in males it is more intense from the onset and remains of about the same order The effects of cortisol on sodium excretion are profoundly different with advancing age in rats of different sexes In males these effects tend to disappear completely In females, on the other hand, they appear earlier and remain of the same order of magnitude

After cortisol treatment we can observe comparable differences in potassium excretion Whereas young males respond with an intense potassium excretion which drops rapidly as the animals grow older, these changes are only moderate in females, potassium excretion remaining high until old age and its onset merely retarded

These age and sex bound differences become particularly clear if we study the variations in the sodium/potassium ratio The sensitivity of the animals to the effects of aldosterone

increases with advancing age, females showing much greater differences than males By way of contrast, sensitivity of the animals to cortisol diminishes with advancing age, females showing here too a greater sensitivity than males

The similarity of the curves of the sodium/potassium ratio for aldosterone and cortisol is also striking and leads us to the problem of (a) the primary and (b) the secondary effects of these substances, and furthermore to the problem of the classification of adrenal steroids on the basis of what has been considered their most important physiological effects

From previous experiments with aldosterone one is inclined to consider as primary effects both sodium retention as a consequence of increased tubular resorption of sodium ion, and potassium excretion as a consequence of the exchange between sodium ions in the tubule cells (Cole, 1957, Stanbury, Gowenlock and Mahler, 1958) Sodium retention remains of about the same order of intensity and duration from youth to old age in both males and females It is concomitant potassium excretion that rises strikingly with advancing age both in males and females

On the other hand, the diuresis induced by aldosterone, which is most apparent in old female animals in the later phases of the experiment, is most probably of secondary origin, its causes lying in the effect of aldosterone on the sensitivity of adrenalectomized animals to endogenous antidiuretic hormone (Gaunt, Lloyd and Chart, 1956)

As for cortisol, its essential effect seems to lie in the very marked potassium excretion which is regarded as running in parallel with its catabolic effects

Its effect on potassium excretion tends to diminish with advancing age, male animals being here more susceptible than females Conversely, the diuretic and sodium excret... pert...

ant

am ... hormone, these properties tend to disappear with increasing age in males but not in females Aldosterone and cortisol tend to induce a greater diuretic

response and concomitantly higher sodium excretion with advancing age, especially in females

This, together with the similarity of the changes in the sodium/potassium ratio induced by aldosterone and cortisol during these experiments, even if the factors of ageing and sex act differently on them, underlines certain similarities of effect in a number of known adrenal steroids which have already been stressed (Meier and Desaulles, 1956, Gaunt and Chart, 1958) Relative dosage, time, age, experimental conditions and different stages of homeostasis are among the factors modifying these similar patterns of effect The relation of homeostasis to the development of the animal organism is too complex to permit of any definite statement We have simply tried to show that the properties of certain hormones may be profoundly affected by such factors as sex difference and increasing age, and that these differences may act in the same or in quite different ways and thus contribute towards a better understanding of pathophysiological changes due to age

Summary

It has been shown that in rats of differing age and sex the sensitivity to the influence of aldosterone and cortisol on urinary electrolyte excretion varies greatly

Whereas age tends to increase sensitivity of the animals to the effects of aldosterone, their sensitivity to cortisol by way of contrast tends to diminish

On the other hand, female animals show a greater responsiveness to these changes than male animals

These results are discussed

Acknowledgement

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DISCUSSION

Adolph Dr Křeček how do you account for what I take to be an absence of [illegible]
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Křeček Yes it was because the pattern of [illegible]
the adr[illegible]

response and concomitantly higher sodium excretion with advancing age, especially in females

This, together with the similarity of the changes in the sodium/potassium ratio induced by aldosterone and cortisol during these experiments, even if the factors of ageing and sex act differently on them, underlines certain similarities of effect in a number of known adrenal steroids which have already been stressed (Meier and Desaulles, 1956, Gaunt and Chart, 1958) Relative dosage, time, age, experimental conditions and different stages of homeostasis are among the factors modifying these similar patterns of effect The relation of homeostasis to the development of the animal organism is too complex to permit of any definite statement We have simply tried to show that the properties of certain hormones may be profoundly affected by such factors as sex difference and increasing age, and that these differences may act in the same or in quite different ways and thus contribute towards a better understanding of pathophysiological changes due to age

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DISCUSSION

Adolph: Dr Křeček, how do you account for ... [illegible]

Křeček: Yes, it was because the pattern of diuresis changes after the administration of vasopressin, so that after ... [illegible]

[illegible]

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Heller That is almost exactly what we found; our age groups were 20–22 and 29–31 days after birth

Borst Has ...
and Dr Křeček ...
are not ...

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time ...
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Desaulles Ours were done very early in the morning

Adolph Did you run controls without the hormones?

Desaulles Every group was run with controls.

Borst Light is not important In blind people the diurnal rhythm remains normal if they are in light during the night and in the dark during the day

Desaulles We cannot cope with every activity, but we did think that light might be one of the problems

Fourman Dr. Desaulles, you drew an analogy between the effects of aldosterone and of cortisol on the excretion of sodium and potassium If one considers the excretion ratio of these two ions in the urine, the effects do appear to be analogous I red ...
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... is not necessary ...

... of cortisol on potassium was secon...
... the tissues

long as they equate these end effe...

Fourman The early rise in potassium excretion with cortisol is probably a cellular effect The results will not be very reliable if you assay a

hormone by a change in Na/K ratios in the urine, when the change is produced by two different mechanisms.

Desaulles: I just wanted to show in this experiment that ages bring changes, and sex too.

Na/K ratios.

Milne I am confused by your statement, Dr Fourman You make

if you spay the female, the changes are much less impressive, but there still remains a great difference between the two sexes I want to stress here a point that is always a little puzzling to me if you spay a female you produce a marked adrenal enlargement, but if you castrate a male the

to become less?

find that in young animals there is very little difference in water diuresis as compared to that in animals 33 days old Between adult animals and 33 day-old ones there is no difference, but between 23 and 33 days there are variable, but statistically significant differences in the excretion of the water load

Heller: That is almost exactly what we found, our age groups were 20–22 and 29–31 days after birth

Borst: Has diurnal rhythm been taken into account by Dr Desaulles and Dr. Křeček? Big differences can arise if the controls and experiments are not done at the same time each day.

Křeček Our experiments and controls were always done at the same time in the morning They were done in summer and in winter, with the same results

Desaulles: Ours were done very early in the morning

Adolph Did you run controls without the hormones?

Desaulles Every group was run with controls

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Desaulles That is my opinion too

Fourman: What strikes me is that pharmacologists are mistaken as long as they equate these end effects of excretion of sodium and potassium, and as long as they speak about the alteration in Na K ratio and use this as a measure of aldosterone effect I think the early effect of [illegible] the effect [illegible] cellular.

Fourman The early rise in potassium excretion with cortisol is probably a cellular effect The results will not be very reliable if you assay a

THE EFFECT OF AGE ON THE ELECTROLYTES IN THE RED BLOOD CELLS OF DIFFERENT SPECIES

M J KARVONEN

Department of Physiology Institute of Occupational Health, Helsinki

Two kinds of age changes may occur in the red blood cells The erythrocytes themselves have a definite length of life which may be determined in various ways, whereas the longevity of "fixed" tissue cells generally cannot be as exactly indicated Thus, as cells erythrocytes may be 'young" or "old" On the other hand, like any other cells of the body, the red cells may be a part of a young or of an old organism

Cellular age

In order to study age changes in the erythrocytes as cells, two principal ways are open One of them is to produce anaemia, e g by bleeding, and thus to stimulate erythropoiesis so that a large proportion of the circulating cells will have been produced within a relatively short period The writer is not aware of any systematic study of the red cell electrolytes throughout the regeneration after acute bleeding In microcytic anaemias of man—which is the type seen also in bleeding anaemia—the concentration of potassium in erythrocytes is lower than normal (Maizels, 1936) In other types of anaemia a change in the opposite direction may occur (Maizels, 1936, Selwyn and Dacie, 1954, McCance and Widdowson, 1956) However changes in the electrolytes observed in any type of anaemia are not necessarily dependent on the age of the erythrocytes, but may be caused by many other factors associated with anaemia

Desaulles. That is a very important point, because it is very well known that if
responsiveness
the following r
and other similar experiments, and at different periods after castration we tested the sensitivity of their sexual adnexa. We observed that what was found by Parkes and Deanesley about 20 years ago is still absolutely valid. You must begin the experiments between two and three weeks after castration, after that the sensitivity diminishes very rapidly. If you wait from one to three months some responses disappear completely, and you need very high dosages of the substance to obtain

but not entirely removed by castration

the red cell electrolytes may change with age The subject was taken up by Hallman and Karvonen (1949) in another species sheep A distinct difference between foetal and adult Finnish sheep was observed, in the sense that the concentration of potassium in erythrocytes was higher in foetal than in adult sheep Fig 1 shows the differences in both

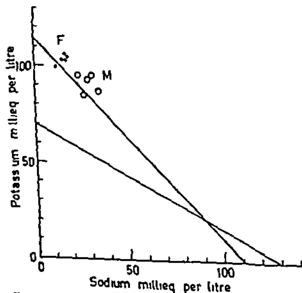


FIG 1 The concentration of potassium and sod[illegible]

sodium and potassium concentrations The sum of the two electrolytes tends to remain constant with age

Widdas (1954) confirmed this observation and found a gradual decrease of the potassium and an increase of the sodium with advancing foetal age

The study by Hallman and Karvonen (1949) brought out another interesting finding In 1898 Abderhalden published

Recently it has been claimed that other methods for studying young or old erythrocytes might be feasible According to Borun, Figueroa and Perry (1957) after centrifugation of blood the bottom layer contains the oldest cells, and the surface the youngest ones An analysis of the different layers has shown that—at least in human adult blood—the packing is closest and the amount of intercellular plasma lowest in the bottom layer, but when the effect of different packing is corrected there is no difference between the sodium and potassium concentrations of the bottom and the surface erythrocytes (Leppanen personal communication) Serial osmotic haemolysis has also been suggested as a means for differentiating young and old erythrocytes (Simon and Topper, 1957) The value of these methods is not yet clear However, the nature of the methods used suggests that changes in the electrolyte metabolism of the erythrocytes may be involved in their ageing though such changes may not necessarily result in differences in the concentration of sodium and potassium

Age of the animal

As a mixed population of different cellular ages erythrocytes are easily available The availability and development of flame photometric analysis have been a stimulus for several investigations of the electrolyte content of the red cells It has been found that in general the sodium and potassium content of the erythrocytes *in vivo* is fairly stable typical of the species, and resistant to many physiological and pharmacological agents However in disease particularly in febrile states, erythrocytes tend to lose potassium and gain sodium in other words, the electrolyte composition of the erythrocytes moves closer to that of plasma

Sheep and other ruminants It may be inferred from results published by Green and Macaskill (1928) and by Wise and co-workers (1947) that the intracellular potassium concentration is higher in the blood of young calves than in that of adult cattle These two papers were the first to indicate that

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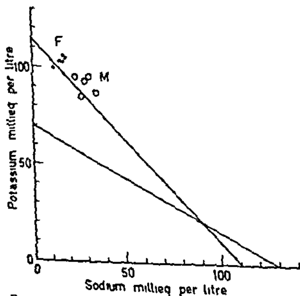


Fig 1 The [illegible]

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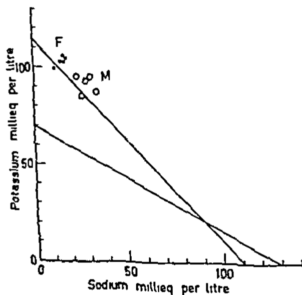


FIG 1 The conc... erythrocytes ... belonging to ... 1949) The c... adult sheep c... ... and ...)

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the first values for the sodium and potassium concentration of adult sheep erythrocytes, and found that they belong to the "low potassium—high sodium" type In 1937 Kerr observed higher potassium concentrations, with a large variation between individual sheep In the determinations of Hallman and Karvonen, the erythrocytes of the Finnish sheep turned out to be—contrary to those of Abderhalden—of the "high potassium—low sodium" type, containing still more potassium than the red cells of Kerr's sheep Sheep erythrocytes thus show a large range of individual variations in the electrolyte composition

The electrolytes are not the only constituents of the red cells in which individual sheep differ The solubility characteristics of sheep haemoglobin obtained from different countries, from different breeds, or from different sheep may also differ (Karvonen, 1949, Karvonen and Leppänen, 1952) It was natural, as a working hypothesis, to connect with each other these differences in the red cell electrolytes and in the type of haemoglobin In the first five samples representing different breeds of sheep, haemoglobin prepared from the low potassium erythrocytes actually showed a crystal habit different from that of the high potassium cells (Karvonen and Leppanen, 1952)

Since these early attempts the red cell electrolytes of sheep have become the subject of intense study The individual differences in the electrolyte composition have been shown to be permanent characteristics (Evans, 1957) The occurrence of different types of red cells in a number of breeds has been studied, and the genetics of the inheritance have been worked out (Evans, 1954, 1957, Evans and King, 1955 Evans *et al*, 1956, Evans and Mounib, 1957)

The application of paper electrophoresis to sheep haemoglobins has shown that though there is a definite association between the electrolytes in the red cells and the haemoglobin, this association is not absolute (Harris and Warren, 1955, Evans *et al*, 1956, Evans, Harris and Warren) On the other hand, the haemoglobin present in the red blood cells has an

influence on the concentration of potassium in the whole blood of both high potassium and low potassium sheep, and thus presumably also on the concentration of potassium in the cells themselves (Evans *et al*, 1956).

The study of individual differences between adult sheep thus shows that the type of haemoglobin is associated with the red cell electrolytes, but that other factors also play a rôle

Haemoglobin changes with age the haemoglobin of a foetus differs from that of

with that of younger animals (Karvonen, unpublished)

The transition from foetal to adult life involves a change of

In sheep, these

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exactly parallel would be a subject of considerable theoretical interest

Other species The effect of age on the electrolyte concentration of red cells has been studied in few other species Remarkably enough, a relationship just opposite to that in ruminants has been found the sodium concentration is higher and the potassium concentration the same or lower in foetal than in adult erythrocytes, at least in man (Hallman, Österlund and Vara, 1954, Österlund, 1955, McCance and Widdowson, 1956), pig (McCance and Widdowson, 1956), and in guinea pig (Widdas, 1954, 1955, Karvonen and Leppänen, unpublished) The concentration of chloride changes in the same direction as that of sodium

Underlying mechanisms

It has been pointed out by Conway (1957) that the smaller a cell, the more work per unit cell volume a "sodium pump" must do in the same environment of plasma or extracellular fluid, in order to keep the intracellular sodium at constant

level A similar conclusion applies to an eventual "potassium pump" The erythrocytes of a foetus are larger than those of an adult With constant activity of the electrolyte pumps an increase in the cell sodium and a decrease in potassium would be expected from foetal to adult life This is the direction of development in the ruminants, but not in the other species examined It is rather questionable whether the decrease in cell size even in the ruminants is an important cause of the changes of the red cell electrolytes

With the aid of *in vitro* studies much progress has been made in elucidating the mechanism of cation transfer across the red cell membrane The application of these methods to the erythrocytes of the foetus suffers from a serious limitation the cells of foetuses (at least human and sheep) haemolyse spontaneously and rather fast *in vitro* To some extent the rate of haemolysis is dependent on oxygen tension high oxygen tension increasing the rate of haemolysis, but haemolysis also occurs at an appreciable rate in blood exposed to nitrogen Haemolysis in human cord blood may also be retarded by administering ascorbic acid to the mothers before delivery, but even so the rate of spontaneous haemolysis remains considerably higher than in adult blood An addition of ascorbic acid *in vitro* is without effect (Raihä, 1950, and personal communication)

Summary

Information on changes in the electrolyte metabolism of individual erythrocytes during their life cycle is meagre However, the claims that young and old cells may be separated with the aid of centrifugation or serial haemolysis suggest that changes in the electrolyte metabolism may be involved in the ageing of the red cells Differences in the actual sodium and potassium concentrations have not, however, been demonstrated

In sheep and cattle the erythrocytes of a foetus contain more potassium and less sodium than those of an adult In man, pig and guinea pig a difference in the opposite direction

has been observed The eventual association of the difference in red cell electrolytes with a difference in haemoglobins and with a difference in cell size is discussed

In vitro studies of foetal erythrocytes and particularly, their interpretation are handicapped by a fast rate of spontaneous haemolysis in foetal blood In man this may be retarded by exposing the blood to nitrogen and/or by administering ascorbic acid to the mother before delivery, but even so the rate of spontaneous haemolysis remains far above that observed in adult blood

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MAIZELS [illegible]

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level A similar conclusion applies to an eventual "potassium pump" The erythrocytes of a foetus are larger than those of an adult With constant activity of the electrolyte pumps an increase in the cell sodium and a decrease in potassium would be expected from foetal to adult life This is the direction of development in the ruminants, but not in the other species examined It is rather questionable whether the decrease in cell size even in the ruminants is an important cause of the changes of the red cell electrolytes

With the aid of *in vitro* studies much progress has been made in elucidating the mechanism of cation transfer across the red cell membrane The application of these methods to the erythrocytes of the foetus suffers from a serious limitation the cells of foetuses (at least human and sheep) haemolyse spontaneously and rather fast *in vitro* To some extent the rate of haemolysis is dependent on oxygen tension, high oxygen tension increasing the rate of haemolysis, but haemolysis also occurs at an appreciable rate in blood exposed to nitrogen Haemolysis in human cord blood may also be retarded by administering ascorbic acid to the mothers before delivery, but even so the rate of spontaneous haemolysis remains considerably higher than in adult blood An addition of ascorbic acid *in vitro* is without effect (Raihä, 1950, and personal communication)

Summary

Information on changes in the electrolyte metabolism of individual erythrocytes during their life cycle is meagre However, the claims that young and old cells may be separated with the aid of centrifugation or serial haemolysis suggest that changes in the electrolyte metabolism may be involved in the ageing of the red cells Differences in the actual sodium and potassium concentrations have not, however, been demonstrated

In sheep and cattle the erythrocytes of a foetus contain more potassium and less sodium than those of an adult In man, pig and guinea pig, a difference in the opposite direction

deer? Deer may have sickle cells, and this is correlated with a certain type of different haemoglobin

Davson I know the camel has ellipsoidal cells

Bull Is anything known about the relative efficiency of the different kinds of red cells with respect to their function of carrying oxygen, in [illegible]

[illegible]

[illegible] Dr Davson, can you comment upon the genetic side of this? You spoke about the sodium pump, what about the difference in haemoglobin?

Davson I cannot relate this at all I do not see why a given type of haemoglobin should be associated with a given electrolyte content.

DISCUSSION

reticulocyte or an erythrocyte it loses the power of accumulation of

has only an anaerobic source of metabolism

usually has a very definite cause and is usually due to the fact that the permeability of the membrane becomes abnormally high and you get this Donnan difference of osmotic pressure being exerted between the plasma and the contents of the cell. Therefore, the most profitable line of research would be to try and get conditions in which you could prevent this haemolysis from occurring.

Milne: Is there any data available on the foetal levels in the cat and the dog, which have this very high sodium content in the erythrocytes?

Karvonen: No, we have none.

Davson: The sheep can have as high a sodium content as the cat, yet

a qualitative idea of the content.

Karvonen: That would be a very interesting thing to do.

absolute

with low

different

slightly

Desaulles: Has not the same effect been described for some kind of

THE DEVELOPMENT OF ACID-BASE CONTROL

E M Widdowson and R A McCance

Medical Research Council Department of Experimental Medicine, University of Cambridge

General Principles (as they apply to adults)

When the body of a healthy person is provided with the diet normally eaten in Europe and the United States, it produces in its metabolism more non volatile anions than cations These "surplus anions" are excreted by the kidney partly in combination with titratable hydrogen ions (the titratable acidity) and partly as ammonium salts The ammonium salts usually account for rather more than 50 per cent of the total If the excess of non volatile anions increases, the pH of the urine falls and the titratable acidity increases, but the excretion of ammonia also increases because a fall in the pH of the urine is *one* of the things which raises the output of ammonia and consequently the percentage of the surplus anions excreted as ammonium salts remains about the same The excretion of ammonium salts is also increased (a) if the pH of the urine is maintained at its lower limits for some ti ... continuous administration of acid or acid forming d ... ncrease in the activity o ... lyse the formation of ammonia and particu ... minase (Davies and Yudkin 1952) (b) By an increase in the acid "load" (Rector, Seldin and Copenhaver 1955) Both (a) and (b) increase the percentage of the surplus anions excreted as ammonium salts and good examples of the effects which may be observed after continuous high dosage are given by Ryberg (1948) As the pH of the urine rises progressively above 6 5 the percentage of the total output of surplus non volatile anions excreted as ammonium salts may also rise and ultimately

Black On the genetic side it is very odd that one gets this scatter right along the line One would think that, according to Mendel one would get segregation at the two ends of the line

I was not clear whether there was an excess of fluid in the red cells In other words, in the foetal sheep or man was there an excess of potassium per litre of red cells? Was there any difference in phosphate content? Differences in phosphate content have been described I think by Prankerd (1955 *Clin Sci* 14, 381) and others in connexion with the sickle cell problem, and I wondered whether that side had been gone into with foetal versus grown up sheep

Karvonen I am afraid I gave a wrong impression when I said the scatter was all along that line There is a very clear concentration at each end of the line but there is also a group in between Within each group, however there is quite a considerable scatter which is due to a permanent individual characteristic of each sheep The statisticians say that there is quite a high intra individual correlation

The foetal cells contain more water than the adult cells In sheep I do not think that any determinations of the phosphate have been done but in man and in pig it has been found (McCance and Widdowson 1956) that the phosphate of the foetal cells is higher

Dawson It must be realized that when red blood cells are analysed, very large numbers are used there may well be differences in concentrations of potassium and sodium amongst the individual ones and they could well fall into groups which would never be discovered Variations in the Na K ratio could be reflections of variations in the proportions of high potassium and low potassium cells which would give a continuous scatter right along the line

on four additional babies on the 7th to 8th day Of the seven babies investigated one week after birth, six were breastfed and the seventh was fed on Ostermilk Samples of blood have been taken from the cord at birth, and from the femoral vein at 48 hours and seven days Urine has also been collected for 24 hours from one child aged eight months and from one aged one year, while six normal men and women have provided 24 hour urine collections to serve as the adult comparisons The urines were collected and stored under toluene Determinations of pH, titratable acid, ammonia, creatinine, phosphate citrate and sulphate have been made on the urine, and the sera have been analysed for creatinine, CO_2, chloride, sodium and potassium

The excretion of surplus anions

Fig 1 shows the millimoles of surplus anions not combined with fixed base (i e titratable acid plus ammonium salts) excreted by the infants on the first, second and seventh days of life and by the older infants A figure for the adults is indicated also All the values are expressed per kg of body weight per day The average pH of the adult urine was 6 or a little over while that of the babies was between 5 5 and 5 8, [illegible]

that of the adults although the pH of the urine was lower, which would have led one to expect a higher rather than a lower anion excretion This low rate of excretion was quite sufficient to maintain the acid base balance of the body, for the serum CO_2 and chloride did not change It is to be attributed to the fact that the urine contains very little phosphate or sulphate at this period (McCance and von Finck, 1947 and see later), owing to the small breakdown of tissue protein (McCance and Strangeways, 1954) By the seventh day the babies were taking nearly 500 ml of breast milk a day, which contained 9 5 g protein or about 3 g/kg, and they were passing about three times as much urine per kg of

reach 100, because above pH 6·5 the excretion of titratable acid falls more rapidly than the ammonia and is extinguished before the excretion of ammonia, which continues at a decreasing rate up to pH 8. This tendency of the percentage to rise as the pH of the urine goes above 6·5 is therefore exaggerated if the urines are titrated, as they mostly are nowadays, to pH 7·4 instead of, as at one time, pH 8.

Dihydrogen orthophosphates are the main buffer acids which can be titrated in a normal adult's urine, but this may not be so in disease if there is a great excess of abnormal organic acids of the right buffer strength in the urine, such as β-hydroxybutyric acid or amino acids. Apart from the phosphates and weak organic acids which contribute by their presence to the titratable acidity, the surplus of non-volatile anions in the urine is very largely due to sulphates, derived from the metabolism of protein (Hunt, 1956). Chlorides are generally balanced by the equivalent amount of fixed base unless calcium or ammonium chloride has been taken to produce a chloride acidosis.

The ability of the kidney to excrete hydrogen ions into the tubules, and so to excrete the surplus non-volatile anions in the way described, depends upon the activity of carbonic anhydrase. Since it has been shown experimentally that the degree to which the pH of the urine can be lowered depends upon the activity of the carbonic anhydrase at any given time, it may be that the lower and well-known limit of urinary pH attainable by a normal person is an expression of the activity of his carbonic anhydrase, but this is merely a suggestion at the moment.

The Newborn Period and Later Infancy

Complete collections of urine from three healthy baby boys have been made for the first 48 hours of their lives, and again over the whole of the 7th–8th day. These babies all passed urine at the moment of birth and this was also collected. Urine passed by two other babies at birth has also been included in the series, and a 24-hour collection has been made

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body weight as the adults Their excretion of surplus anions, sulphates among them, per kg, had reached the adult level although they were still excreting little or no phosphate The pH of their urine was a little higher than it was on the first two days, and the increased volume may have been one reason for this (McCance and von Finck, 1947, Hungerland, 1957)

At eight months to one year of age the babies excreted more

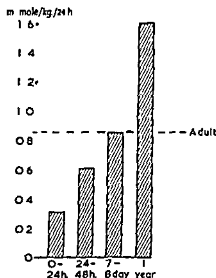


FIG 1 Surplus anions (not combined with fixed base) excreted by babies during the first week and at 8 months to 1 year of life

surplus anions per kg of body weight than the adults This is explainable by the high intake and metabolism of protein per kg of body weight at this time of life A child of one year consumes about 3 5 g protein per kg, which is two to three times as much as an adult per kg, and only 8 or 10 per cent of it is used for growth in contrast to the 50 per cent or so retained in the neonatal period The phosphates and the cystine and methionine in the milk and other protein foods were probably the main sources of the surplus anions

Fig. 2 shows the percentage of the surplus anions excreted with ammonia. For this it is possible to give a figure for urine which was formed *in utero* and passed at the moment of birth and which had a pH of over 6. It will be observed that, although the pH of the urine passed at birth was higher than that of the urine passed afterwards, the percentage of the surplus anions excreted with ammonia was also very high before birth, and of the order to be expected in adults with

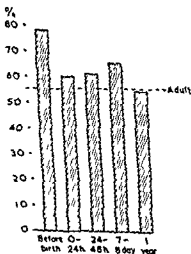


FIG 2 Percentage of the surplus anions excreted as ammonium salts.

very acid urines after taking large doses of ammonium chloride for some days The percentage of the surplus anions excreted with ammonia in the first 48 hours and on the seventh day of life has also tended in our series to be higher than that in the urine passed by adults This is probably because the babies' urine contained so little phosphate, and consequently the titratable acidity was low in relation to the total amount of surplus an [illegible]

of [illegible]

th [illegible]

[illegible] ... Work

body weight as the adults. Their excretion of surplus anions, sulphates among them, per kg., had reached the adult level although they were still excreting little or no phosphate. The pH of their urine was a little higher than it was on the first two days, and the increased volume may have been one reason for this (McCance and von Finck, 1947; Hungerland, 1957).

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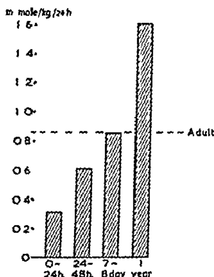


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t is evidently lower even than the excretion of ammonia By one year of age the glomerular filtration rate/kg had risen above that of adults (McCance and Widdowson, 1952), and more ammonia and surplus anions per kg were being excreted (see Figs 1 and 3), the amount of ammonia excreted per ml of glomerular filtrate was near the adult level

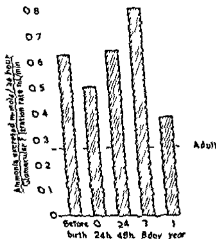


FIG 4 The ratio of the ammonia excreted (m mole/24 h) to the glomerular filtrat on rate (ml./min)

The nature of the titratable acidity

Fig 5 shows the excretion of titratable acid per kg of body weight by the babies and the adults The amount excreted was low during the whole of the first week, but it was rising even though the urine still contained no phosphates The high excretion at a year is again related to the high intake of protein at that age

Fig 6 shows the percentage of the titratable acidity due to phosphate in the urine of an adult and in the urine of a breast fed baby in the first week of life In the adult the percentage depends upon the pH and, since the pH of the urine passed

which has been done on kidney slices *in vitro* (Robinson, 1954), and on renal glutaminase and ammonia production (Hines and McCance, 1954) goes to show that, weight for weight, the kidney of the newborn of other species contains less glutaminase and produces less ammonia than that of the adult Fig 3 shows that the total amount of ammonia excreted per kg of body weight was in fact small in the first two days, but that by a week, when the baby was taking in three times as much protein as the adult per kg of body weight, it had risen above the adult level The ability to form ammonia in response to an acid load in the first day or two of life has not yet been

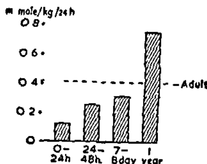


FIG 3 The amount of ammonia excreted

studied in man, but Cort and McCance (1954) found it to be smaller in puppies two days old than in adult dogs The matter requires further investigation

Fig 4 shows the excretion of ammonia in millimoles/24 hours divided by the glomerular filtration rate (as measured by the endogenous creatinine clearance) in ml /minute It was possible to calculate this ratio for the urine passed at birth, even though the rate of urine flow before birth was not known, because the two functions being compared are both expressed in terms of rates of urine secretion The excretion of ammonia was high *in utero* and in the newborn period in relation to glomerular filtration rate The glomerular filtration rate at this time of life is very low by adult standards, and if the endogenous creatinine clearance is a true measure of it,

more than the amount excreted by the adults in this series n so far as citric acid may be regarded as a product of the aetabolism of the kidney it cannot be classed as a surplus mon although it contributes to the titratable acidity.

It is well known that infants on cows' milk mixtures have a ugher concentration of inorganic phosphorus in their serum than breastfed infants, they excrete phosphates by the seventh day of life, and the phosphate organic acid relationship is of the adult pattern, as it is also in the urine of infants eight months to one year of age

Foetal Life

In the uterus the acid base balance of the whole conceptus is regulated ultimately by the mother's lungs and kidneys, but the foetal kidneys, membranes and placenta act as intermediaries

Urine has been taken from the bladders of five human foetuses aged 10–20 weeks It has always been found to be hypotonic, due mainly to very low concentrations of sodium and chloride It appears to resemble the urine formed *in utero* and passed at term which has been better investigated and described elsewhere (McCance and Widdowson, 1953, Hanon, Coquoin Carnot and Pignard, 1955, 1957)

The pig has a gestation period of about 120 days Between the 20th and 60th day there is a rapid expansion in the volume of allantoic fluid The sac containing the fluid has free connexion with the kidney through the urachus and bladder. Its membranes also participate in exchanges with the mother Table I shows the composition of the fluid at 20 days, 45 days and 60 days At 45 days both mesonephros and metanephros are functional, but the former is becoming less so The

[illegible] greatly so that from 45 days it is only one half or one third that of foetal serum (McCance and Dickerson, 1957) This fall in osmolar concentration is due largely to a fall in the concentration of sodium and chloride The

by the present series of adults was higher than that of the newborn infants, the value for adults shown in Fig 6 (70–80 per cent) has been taken from Gamble (1942) Phosphates accounted for a very small fraction of the titratable acidity of

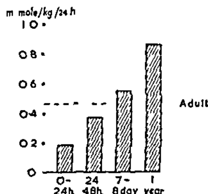


Fig 5 The excretion of titratable acid

the infant's urine, which is due to the fact, already mentioned, that the urine of breastfed infants contains so little phosphate at this time of life

Investigations are being made on the organic acids in the

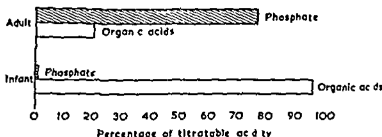


Fig 6 The proportion of titratable acid due to phosphates and organic acids in the urine (pH 5 5 6 0) of adults and infants

urine during the first week of life *Citric acid is one of the* major constituents, and on the seventh day the breastfed babies were found to be excreting 33 mg citrate/kg body weight/24 hours (Stanier, personal communication) This

hoped to extend the study to later stages of gestation, to other membranes and to glutaminase.

before birth, or whether the fluid has been made hypotonic and acid by the action of the membranes themselves Small amounts of fluid have been removed from the bladders of foetuses at 45 days and it is probably possible also to withdraw fluid from the large mesonephric duct, so that this problem may be soluble without recourse to large scale experimental veterinary obstetrics Should it turn out that the composition of the fluid is being altered by the membranes, their activities may contribute materially to our ideas about the function of the renal tubules

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[illegible]

concentration of potassium does not fall in the same way, and the concentration of calcium rises This calcium appears to be held in solution by citric acid (Economou Mavrou and McCance, 1958)

The fluid at 45 days has been found to have a pH between 5 5 and 6, and a titratable acidity of about 10 m equiv /litre The fluid contains ammonia and ammonia appears to account for about 25 per cent of the titratable acid plus ammonia found in it The concentration of phosphates is always small, and the acidity is almost entirely due to carbonic acid The

Table I

THE WEIGHT OF THE FOETAL PIG AND THE VOLUME AND *COMPOSITION OF ITS ALLANTOIC FLUID*

Foetal age	*20 days*	*45 days*	*60 days*
Weight of foetus	0 1 g	20 g	100 g
Volume of allantoic fluid	5 ml	110 ml	350 ml.
Composition of allantoic fluid			
Osmolar concentration m osm /l	256	120	92
Urea m mole/l	3 1	8 4	10 3
Chloride m-equiv /l	69	30	18
Sodium	114	13	14
Potassium	14	8	6
Calcium mg /100 ml	6	30	—
Inorganic phosphorus mg /100 ml	9	6	—

pH rises quickly if the fluid is shaken or even if it is left in a tube exposed to the air, and it was found necessary to collect and analyse the fluid out of contact with air Lutwak Mann and Laser (1954) found no "bicarbonate" in pig's allantoic fluid at 20 days' gestation but there is no doubt about the presence of carbonic acid at 45 days

Further investigation has confirmed the fact, first noted by Lutwak Mann (1955), that the chorioallantoic membrane contains carbonic anhydrase At 45 days the allantoic fluid itself also had some carbonic anhydrase activity On the basis of material from three pregnant pigs the activities of carbonic anhydrase may be given as foetal kidney +++, chorioallantoic membrane ++, allantoic fluid + It is

the major organic acid constituent of the urine which is passed immediately after birth In addition urine passed during the first 24 hours of life contains malic acid, glycolic lactic, β hydroxybutyric, succinic, and α ketoglutaric acids, but not aconitic acid With this method one can detect a minimum of 20 μg of each of these organic acids

Adolph Is there any appreciable accumulation of organic acids in the newborn during the first week of life? At this stage the individual is very insensitive to the hydrogen ion concentration changes as far as the breathing is concerned and I was wondering whether it is also insensitive as far as excretion is concerned

Zweymuller We are now working on the detection and identification of the organic acids found in the urine of normal newborn babies and the next problem will be to identify those found in the urine of hypoxaemic newborn babies

Karvonen Did you find any pyruvate or does it come out with this method?

Zweymuller We have not found a pyruvic acid spot, but we have not added pyruvic acid to the urine so we do not know exactly where the spot should appear on the paper

Karvonen I understand that increased excretion of pyruvate has been found during the first few days of life (Taliqvist H (1952) Thesis, Hameenlinna)

Zweymuller There is an interesting paper about some work on the output of organic acids in potassium depletion in which pyruvic acid lactic acid α ketoglutaric acid and citric acid were estimated but this was done on normal adults (Evans *et al* (1957) *Clin Sci*, 16, 53)

Fourman The hydrogen ion in the allantoic sac must come from somewhere it cannot be manufactured It must come in the end from the mother and since she cannot manufacture the hydrogen ion it must ultimately come from her diet So what happens if you feed alkali to the mother pig?

Widdowson We have not tried that

Milne It is well shown in your paper Dr Widdowson, how the newborn baby copes with its normal environment I would agree that the organic acid level especially that of citrate, is proportionally much higher than in the adult Obviously in assessing the efficiency of the kidney particularly in excreting an acid load one must give it a maximum challenge and though I see the difficulties of this in human experimentation it would be extremely interesting to do this in the newborn animal There seem to be two separate aspects of excretion of acid by the kidney One is the ability of the kidney to excrete a maximum amount of hydrogen ion per day and clearly that can only be assessed by giving a prolonged acid load The other is the ability of the kidney to maintain a hydrogen ion gradient between urine and plasma, in other words the production of a minimum urinary pH I would be very interested in having data on whether the minimum pH of adult urine is similar to the minimum pH of newborn urine whether the ammonia excretion can increase on prolonged acid ingestion proportionally to that of the adult,

DISCUSSION

Zweymuller The identification of organic acids in urine by paper

Nordmann and co-workers (1954 *C.R. Acad Sci*, *Paris*, 238, 2459), and Fig 1 demonstrates the position on a two-dimensional descending chromatogram of some non volatile, water soluble organic acids which

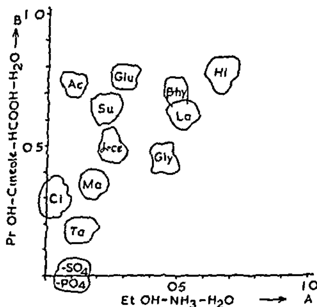


Fig 1 (Zweymüller) The position of some organic acids in the urine of a normal adult on a two dimensional descending chromatogram

Ci = Citric acid, Ta = Tartaric, Ma = Malic
Gly = Glycolic, α ce = α ketoglutaric, Su = Succinic,
Ac = Aconitic, Glu = Glutaric β hy = β hydroxybutyric, La = Lactic, Hi = Hippuric

injections

McCance The lower limits might be due to the activity of carbonic anhydrase having a ceiling in the human kidney We know that if the carbonic anhydrase is defective the lowering of pH is correspondingly limited

and finally whether this very large citrate output in the newborn shows the same tremendous lability to acid base effect as it does in the adult, in whom it can be reduced by quite small doses of acid or increased by alkalinization, say by sodium bicarbonate

Widdowson We have not yet given an acid load to newborn babies, [illegible]

The difficulty in an animal which is developing very rapidly is to separate the effects of several days' administration of an acid forming drug and [illegible]

altering the pH of the urine upon the excretion of citrates in the newborn baby.

Scribner We carried out some experiments in rats which seem to indicate that the amount of citrate in the urine depends on the kidney tissue level of citrate rather than on the blood citrate level After intraperitoneal injection of eithe [illegible] citrate increases 10- to 20 fol [illegible] increases two to threefold I [illegible] response to intraperitoneal [illegible] the blood level rises nearly 100 per cent, but there is little or no increase in either kidney tissue citrate or urinary citrate We concluded from these experiments that the level of citrate in the urine under these conditions is determined by the citrate level in the renal tubular cell and is independent of the amount of citrate filtered through the glomerulus

McCance Dr Milne, what determines the lower limits of pH which the human and other kidneys can achieve?

Milne. I think this can only be answered conditionally First, one must state the stimulus, and secondly one must state the conditions of the kidney at that moment Ammonium chloride has been used as the usual stimulus and I think no one has ever produced a pH of human urine below 4 4 by that method, but other stimuli seem able to produce a considerably lower pH The experiments of Schwartz, Jenson and [illegible] infused [illegible] clearly, [illegible] stimulus, and indeed this agrees in the rat It is very difficult to produce a highly acid urine in rats by most experiments When it is given ammonium chloride the rat seems to be able to keep up with the ammonium intake and puts out ammonium chloride in its urine almost as quickly as it is either injected or taken in the drinking water But an

have not shown a potassium deficiency as in muscle, but they have shown a fall in intracellular bicarbonate and therefore presumably a fall in intracellular pH These experiments have not, as far as I

possibly greater

Shock The histological structure in Prof Wallace's potassium deficient animal wh[illegible]

looking muscle fibres in Prof Wallace's animal than we see in the

[illegible] animals were presumed to look frayed and woebegone Subsequent experiments showed that dietary deficiencies and alterations could produce similar changes [illegible]
of young
older anim
in the yo
result of
basic questions of what is adequate nutrition of a cell and how can it be maintained

Wallace What is old and what [illegible]
quite young while

Shock To me a
when I talk of an
least is at an age

Wallace Young
logical changes in [illegible] muscle These changes can be almost completely reversed in as little as 36–48 hours after potassium administration. The lesions in cardiac muscle do not show this rapid type of healing It would be interesting to see if your old rats have a slower repair time Dr Hingerty has already mentioned that older rats chemically repair potassium deficiency more slowly than do the young ones

GENERAL DISCUSSION

Wallace: I should like to present a problem that arises when one attempts to interpret chemical analysis of tissues from deficient animals in terms of histological appearance. Skeletal muscle taken from potassium-deficient animals is low in potassium, high in sodium, high in its content of basic amino acids and probably low in bicarbonate content When the muscle is examined histologically one sees apparently normal cells lying side by side with grossly abnormal cells. Which cells account for the chemical abnormalities? I have wondered if a cell can tolerate any deficit at all. Possibly, for the cell, it is an all-or-none phenomenon Does a tissue as a whole become deficient in a sort of quantum fashion, cell by cell rather than by an over-all shared process by all of the cells? Is it not necessary to get down to a truly cellular level to further our understanding?

Fourman. May I add to Prof Wallace's problem? The kidney and the heart show the morphological changes of potassium deficiency before the other tissues. These two tissues, when they are analysed in animals that have been made deficient in potassium, do not as a rule show chemical evidence of potassium deficiency. I suppose they do if you carry the deficiency far enough but as a rule they do not. It has always been a puzzle to me why two tissues that have a normal potassium content are the first tissues to show a potassium abnormality. These two tissues are also ones that never rest, in the way muscles do, and one wonders whether the fact that their function requires the maintenance of a normal potassium content, with the demand on the metabolic energy of the cell that this entails, carries the seeds of their own destruction

Wallace The analyses of Orent-Keiles and McCollum do show deficits of potassium in cardiac muscle taken from deficient rats (1941. *J. biol. Chem*, 140, 337). However, most workers have not shown the same thing

Black: Jean Oliver and co-workers (1957 *J exp Med.*, 106, 563) have done work on the localization of the morphological defect in the nephron of potassium depleted animals, and this seems to be limited to the proximal and the collecting tubules. Dr Fourman's difficulty may not be so real if the lesion is as sharply localized as that. With analysis of the whole kidney that may just be a failure to detect a limited local deficiency of potassium.

Milne: Part of the difficulty may be this. is not the necrosis or degeneration in the cell possibly due to the fall in intracellular pH, not primarily to potassium deficiency? I agree that kidney analyses

have not shown a potassium deficiency as in muscle, but they have shown a fall in intracellular bicarbonate, and therefore presumably [illegible]

possibly greater

Fourman Yes, unless you think as I did, that the fall in intracellular pH is a result of the fall in intracellular potassium

[illegible]

has prepared from our material, which show a reduction in potassium content of the total muscle mass There were fewer nice looking muscle fibres in Prof Wallace's animal than we see in the [illegible]

ago there was quite a flurry about the electron microscopic studies of mitochondria In such pictures the mitochondria from cells of old animals were presumed to look frayed and woebegone Subsequent experiments showed that dietary deficiencies and alterations could produce similar changes in the mitochondria taken from cells of young animals If the few cellular changes we can observe in older animals can be produced by nutritional and dietary alterations in the young ones, it is possible that these 'age changes' are the result of chronic malnutrition of the cells This brings us to the basic questions of what is adequate nutrition of a cell, and how can it be maintained

Wallace What is old and what is yo[illegible]
quite your

Shock [illegible]
when I ta
least is at

Wallace [illegible] made potassium-deficient do show morphological changes in skeletal muscle These changes can be almost completely reversed in as little as 36–48 hours after potassium administration The lesions in cardiac muscle do not show this rapid type of healing It would be interesting to see if your old rats have a slower repair time Dr Hingerty has already mentioned that older rats chemically repair potassium deficiency more slowly than do the young ones

Kennedy: Morrison and Gordon (1957. *Fed Proc*, 16, 366, and personal communication) have reported that a 24 month old rat starved of food but not water for 24 hours loses far more urea, creatinine and potassium than a young one of comparable weight So there is a state of incipient potassium deficiency We have also [illegible]

[illegible]

al, (1952). *J. Geront*, 7, 351; Bogdonoff *et al*, (1953, 1954) *J. Geront*, 8, 272; 9, 262, Watkin *et al*, (1955) *J. Geront*, 10, 268) We consistently found that the older individuals, when given good protein intakes that resulted in positive nitrogen balances, retained potassium in excess of the theoretical amount required for the nitrogen retained A good deal of this, I am sure, may be due to cumulative analytical errors, but it has always seemed to me that the older animal will work himself into a potassium deficiency if given the opportunity.

Black Is not some of our difficulty here due to the limitations of morphology? If we take as our criteria of morphological change the [illegible]

[illegible]

how we can expect morphology to decide its aetiology

Talbot When you use the term 'potassium deficient', Prof Wallace, do you wish us to think simultaneously about the correlated fact of the cellular sodium excess? Cellular sodium intoxication may actually be the provocative factor under some circumstances

Wallace Sodium excess is usually a corollary but not always Some cation, it would seem, must replace the deficit Basic amino acids have been shown to increase in potassium deficient tissues as well as sodium

Talbot We have just done some experiments where the absolute [illegible]

[illegible]

[illegible]

by your work (Conway, E J, and Hingerty, D J. (1948) *Biochem J*, 42, 372) Unlike you we found that sodium was lost simultaneously with a gain of muscle

potassium to [illegible]
(1955) *An* [illegible]

Swyer T[illegible]
and other [illegible] what is
basis or ma[illegible]
the true sex [illegible] androgens and oestrogens, or
the ratio of [illegible] two sex hormones, or is it in fact due to some characteristic which depends upon the presence of one X or two X chromosomes?

Kennedy It is probably something to do with species, but the differences in size and growth between the castrate cockerel and the castrate hen, and the same sort of thing in male and female castrate rats, are very well known, and there is obviously a genetic difference in the subsequent behaviour of the neonatal castrate Some of the early theories of ageing depended on body size, and one wonders how much actual size, or organ development and growth as such, rather than sex alone, affects the matter The kidney of the male castrate rat, even though it is castrated very young, is a much bigger organ and in some senses therefore, is a more developed or older organ than that of a female rat Purely structural factors may determine some of the differences in what I think you call end-organ responsiveness

Swyer Is castration even shortly after birth early enough? After all the foetal testis has a very important role to play and intra-uterine castration might avoid this difficulty

Desaulles That might possibly be helpful in determining the role of the X zone It is hard to imagine how the interrelationship between pituitary, adrenals and gonads acts just at the beginning of life in the animal

Milne Is the control to the castrate male a spayed female?

Desaulles They are quite different—that is the annoying point

Kennedy When he discussed renal function Dr Shock pointed out that there was some similarity between the old and the young kidneys in their inability to sustain water diuresis and so on It has been shown (Smith H (1951) The Kidney, Structure and Function in Health and Disease New York Oxford University Press) that if you take an animal of intermediate age and remove one of its kidneys and half the other then the initial response, at least, is a great diminution in water diuresis which may take four weeks to be restored to about two-thirds normal This may suggest that the period during which the major changes in the newborn develop is during the unfolding of the anlage of the kidney, senescence in most animals that have been studied similarly involves a loss of structural units So again, simply the amount of end organ which is there may be the important thing, apart altogether from what is called the endocrine climate

Kennedy: Morrison and Gordon (1957. *Fed Proc.*, 16, 366, and personal communication) have reported that a 24 month old rat starved of food but not water for 24 hours loses far more urea, creatinine and potassium than a young one of comparable weight. So there is a state of [illegible]
found [illegible]

Shoc[illegible]

[illegible]
tently found that the older individuals, when given good protein intakes that resulted in positive nitrogen balances, retained potassium in excess of [illegible]
A good deal [illegible]
errors, but [illegible]
work himself into a potassium deficiency if given the opportunity.

Black: Is not some of our difficulty here due to the limitations of morphology? If we take as our criteria of morphological change the fact that the tissue 'looks bad' or 'looks moth eaten', then we are not going to get anywhere in deciding the cause of this change. You can hardly expect a cell to have a signpost saying 'I am too old', or 'I am [illegible]
how [illegible]

Ta[illegible]
Wallace, do you wish us to think simultaneously about the correlated fact of the cellular sodium excess? Cellular sodium intoxication may actually be the provocative factor under some circumstances.

Wallace. Sodium excess is usually a corollary but not always. Some cation, it would seem, must replace the deficit. Basic amino acids have been shown to increase in potassium deficient tissues as well as sodium.

Talbot: We have just done some experiments where the absolute [illegible]
[illegible]
had cellular sodium intoxication that showed all the symptoms commonly considered characteristic of marked potassium deficiency.

Hingerty: Prof. Wallace, when you restored the potassium, morpho[illegible] right in 30 hours. Did you [illegible]
[illegible] our work (Conway, E. J., [illegible] 42, 372). Unlike you we found that sodium was lost simultaneously with a gain of muscle

THE RÔLE OF THE KIDNEY IN ELECTROLYTE AND WATER REGULATION IN THE AGED

N W Shock

Gerontology Branch National Heart Institute National Institutes of Health PHS D.H.E & W Bethesda and the Baltimore City Hospitals Baltimore Maryland

The kidney is the first line of defence in maintaining appropriate concentrations of water and electrolytes in the internal environment of all the cells in the body Although there are other avenues through which salts and water may be lost from the body, and other factors which may enter into the regulation of concentrations in local areas, it is the kidney which carries the major burden of electrolyte and water regulation The kidney responds to a multitude of stimuli and is blessed with large reserve capacities It is the purpose of this report to describe briefly some of our findings with regard to age changes in renal function, to discuss the possible mechanisms of these changes and to discuss their relation to the maintenance of certain physiological constants in the aged

In order for the kidney to serve its functions of regulating water and electrolyte concentrations, as well as the volume of extracellular fluid blood must be delivered to it in adequate amounts glomerular filtrate must be formed, and the tubular cells must selectively reabsorb and excrete substances in accordance with a variety of stimuli to which the kidney must respond The application of clearance techniques makes it possible to assess the nature of age changes in discrete renal functions The studies to be reported are based on ambulatory male subjects between the ages of 20 and 90 years who were found to be free from clinical evidence of renal disease as judged by clinical laboratory tests and medical history All

Adolph: I wish the structural picture agreed so well with the physiological response to water loading *First of all, when you take out* one kidney from, say, a middle aged rat, you do not reduce the water diuresis much—it is more often a reduction of 20 per cent than of 50

available, as far as anatomical studies show, is about 50 per cent of *that in the adult, and yet the diuresis may only be 10 per cent of the* adult's. As the diuresis develops in intensity with age, it gets far

measured,

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(1958) *Amer*

clearances

Desaulles: When a heminephrectomized animal is submitted, eight or ten days after operation, to a physiological saline load of about

is given out

volume which damages certain nephrons in several different ways The resulting morphology may be very various but the functional

with a failure

tioning

re right

Although there is a substantial variation between subjects at any given age, the trend is highly significant *

The age decrement in glomerular filtration rate, as measured by standard inulin clearance, is shown in Fig 2 The regression of inulin clearance with age is expressed by the equation $Cl_{In} \approx 153\ 2 - 0\ 96 \times$ age (in years) The average decline over the age span 20–90 years was 46 per cent in this instance †

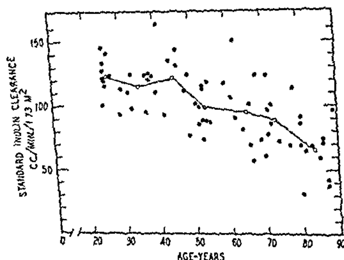


FIG 2 Change in standard inulin clearance or glomerular filtration rate with age O——O average values ml filtrate/min./1 73sq m body surface area.
(From Shock 1952)

The fall in glomerular filtration rate is closely associated with the fall in plasma flow so that the filtration fraction, calculated as ratio of inulin clearance to the diodrast clearance, shows only a slight increase with age (Fig 3)

* In a different sample of subjects in whom renal plasma flow was estimated from PAH (p-aminohippuric acid) clearance (Watkin and Shock 19[illegible]) the

subjects were selected only after a thorough history and physical examination which excluded recent or remote renal diseases, cerebrovascular accidents, coronary artery disease, syphilitic or rheumatic heart disease, hypertension, or any recent alterations in body weight. All tests were carried out under basal conditions and subjects were hydrated with 600–800 ml. water, given orally 1–2 hours before the test, and 200 ml. water were given at half hour intervals during the

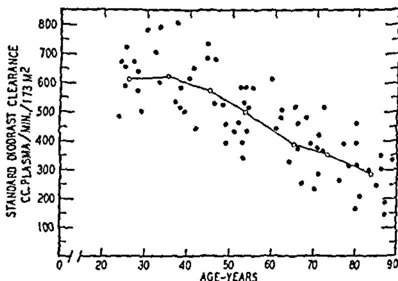


Fig. 1 Change in standard diodrast clearance or effective renal plasma flow with age O———O average values ml plasma/min/1 73 sq m body surface area
(From Shock 1952)

test. The constant infusion method was followed, and four clearance and four Tm periods of 10–14 minutes each were taken according to the method of Smith, Goldring and Chasis (1938). Fig. 1 shows the age change in effective renal plasma flow as estimated from diodrast clearance (Shock, 1952) Between the ages of 20 and 90 years there was a decline in the effective renal plasma flow amounting to approximately 53 per cent. The regression equation relating the diodrast clearance to age is: $Cl_D = 840 - 6\ 44 \times \text{age}$ (in years).

The maximum capacity of the renal tubule to excrete diodrast also diminishes with age Fig 4 illustrates the results of this test in the subjects studied The average diodrast Tm fell from 54 6 to 30 8 mg iodine/1 73 m ²/min between the ages of 20 and 90 years This represents a reduction of 43 5 per cent The regression equation relating diodrast

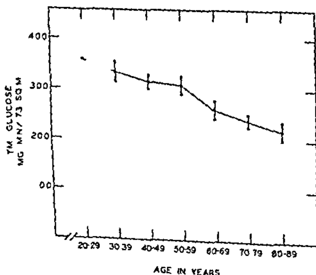


FIG 5 Decrease in maximal tubular reabsorptive capacity with age The slope is drawn to connect the mean values for each decade The vertical lines represent ± one standard error of the mean while the open circles define the lim ts of ± one standard deviation of the distribut on.

(From Miller McDonald and Shock 1952)

Tm to age is $Tm_D = 86\ 7 - 0\ 40 \times$ age (in years) * The reabsorptive capacity of the renal tubular epithelium for glucose also shows a comparable diminution with age, as shown in Fig 5 The average glucose Tm fell from 328 to 223 mg glucose/1 73m ²/min between the ages of 30 and

* The max mum excretory capac ty for PAH shows the following regression on age $Tm_{PAH} = 120\ 6 - 0\ 865 \times$ age (Watkin and Shock 1955)

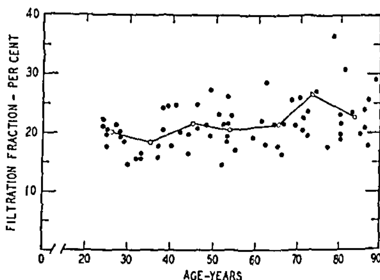


Fig 3. Change in filtration fraction with age O———O average values, per cent of plasma filtered
(From Shock, 1952)

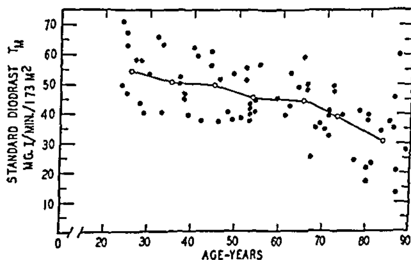


Fig 4 Change in standard diodrast Tm with age O———O average values mg diodrast iodine/min /1 73 sq m body surface area.
(From: Shock 1952)

Landowne and Shock, 1955), as shown in Fig 8, a portion of the reduction in renal plasma flow must be attributed to a reduction in total blood flow However, in experiments to be reported later calculations show that the age reduction in renal blood flow is proportionally greater than the reduction in cardiac output

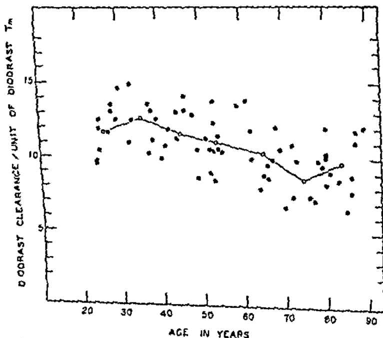


Fig 7 Change in effective renal plasma flow per unit of diodrast Tm
O——O average values
(From Shock 1952)

Other experiments have shown that the reduction of effective renal plasma flow in the aged cannot be ascribed to permanent structural changes in the renal vascular bed (McDonald, Solomon and Shock, 1951) Previous studies have shown that the administration of a pyrogen to young people

90 years The regression equation is $Tm_a = 432.8 - 2.604 \times$ age (in years) The maximum capacity for both a reabsorptive and excretory mechanism in the renal tubules showed approximately the same percentage decrement with age

The average inulin clearance per unit of Tm remains constant between the ages of 20 and 90 years (Fig 6) This finding lends support to the hypothesis that a nephron loses its function as a unit In contrast, the diodrast clearance per

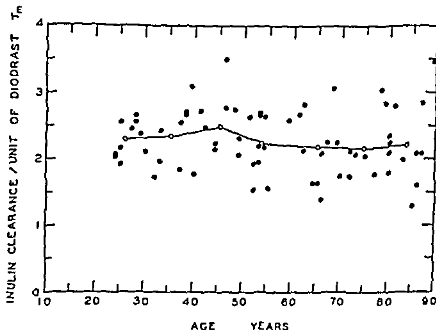


Fig 6 Change in rate of glomerular filtration per unit of diodrast Tm ○——○ average values

(From Shock 1952)

unit of Tm decreases from an average value of 12 6 at age 30-39 to 9 7 at age 80-89 (Fig 7) This steady decline in the effective renal plasma flow per unit of tubular excretory capacity indicates that the average amount of blood delivered to each tubule, and by implication each nephron declines with age Since we have been able to demonstrate a significant reduction in resting cardiac output with age (Brandfonbrener,

average of 20 subjects in each age group, are shown in Fig 9 From the three curves at the bottom of the ... altho ... effect of the pyrogen in

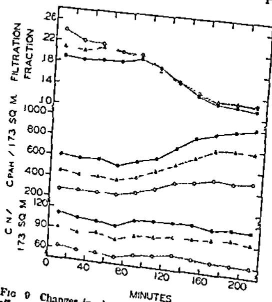


FIG 9 Changes in glomerular filtration rate (C_{In}), effective renal plasma flow (C_{PAH}), and filtration fraction during the pyrogen reaction. Fifty million killed typhoid organisms were injected intravenously at 0 time O——O mean values for 14 subjects aged 70–85 years (O group) Δ——Δ mean values for 20 subjects aged 50–69 years (M group) ●——● mean values for 20 subjects aged 20–40 years (Y group)
(From McDonald Solomon and Shock, 1951)

either the young, middle, or old subjects The three curves in the centre of the graph show clearly that, beginning about 80 minutes after the administration of the pyrogen, there was a slow continuous rise in renal plasma flow in all groups of subjects Although the mean absolute increases were greater for the young than for the old group, where increments were

results in a marked increase in effective renal plasma flow. In order to assess age changes in the ability of the renal vascular bed to dilate, glomerular filtration rate and renal plasma flow

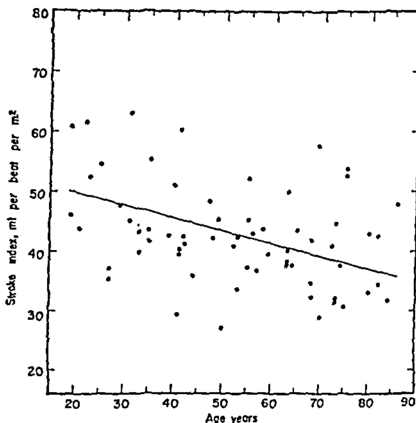


Fig. 8. Stroke output per sq m. surface area versus age. Each point represents the average of two measurements in 40 subjects, of three measurements in four subjects, and a single measurement in 14 subjects. The line indicates the simple linear regression for the data.

(From: Brandfonbrener, Landowne and Shock, 1955).

were measured in young, middle-aged, and old subjects following the intravenous administration of 50,000,000 killed typhoid organisms (0·5 ml. typhoid-paratyphoid A and B vaccine). The results of these experiments, based on the

flows, oral fluid intake was supplemented by the intravenous administration of 5 per cent dextrose in distilled water, in which appropriate quantities of inulin and sodium amino hippurate had been added at the rate of 8 ml /min by a constant infusion pump Twenty nine adult males, ranging in age from 26 to 86 years, served as subjects The total sample was arbitrarily divided into three age groups young (no = 9, age range from 26–45) middle (no = 10, age range from 46–65) and old (no = 10, age range from 66–86) After three control collection periods, 0 05 milliunits pitressin/kg body weight was administered intravenously Subsequently, six consecutive urine collections, each of 12 minutes duration, were made During the control periods, the average urine flow for the young subjects was approximately 14 ml /min , middle aged 11 ml /min and old subjects, 10 ml /min The urine/plasma (U/P) inulin ratio was calculated as an index of water reabsorption The results of this experiment are shown in Fig 10, where the U/P inulin ratio was plotted against the urine collection period During the control periods the U/P inulin ratios were approximately 10 for all three age groups Following the administration of pitressin, prompt antidiuresis was noted in all three groups Peak antidiuresis and peak concentration of inulin were observed in all three age groups during this period which was 12–24 minutes after pitressin As indicated in Fig 10, there was a marked age difference in the antidiuretic response to this standard stimulus The young subjects showed the maximum response and the old subjects showed the minimum In Fig 11 [illegible]
tubular resp [illegible]
is shown C [illegible], and the regression of the concentration on age was described as U/P inulin = 162 − 1 6 × age (in years) Although the administered pitressin resulted in a rise of blood pressure, it averaged only 10 mm at two minutes after injection and fell to control levels within five minutes These experiments indicate that, in the older individual there is an impairment in the

expressed as percentages of the base line values, the rise in renal blood flow for the young, middle, and old groups was 76, 86, and 91 per cent respectively As shown by the upper three curves, the filtration fraction diminished markedly in all subjects, indicating a fall in effective filtration pressure, which would result from a greater vasodilatation at the efferent than at the afferent side of the glomerulus if there were no change in blood pressure Actually, the diastolic blood pressure dropped slightly in the middle and old groups, but remained constant throughout the reaction in the young group At the height of the reaction the differences in the filtration fraction observed under resting conditions, completely disappeared The small absolute changes in renal plasma flow in the older subjects following pyrogen, are consistent with the anatomical findings of a progressive decrease in the number of glomeruli in the aged kidney (Moore, 1931) On the other hand the time of onset and the percentage increase in renal plasma flow were similar in the different age groups Consequently it must be concluded that the responsiveness to pyrogen of the vascular elements remaining in the aged kidney is not qualitatively different from that in the young kidney It is inferred from these experiments that the renal arterioles in the aged kidney are capable of dilating and that in the resting state there is a functional vasoconstriction of the afferent arterioles in the aged which under resting conditions diverts blood from the kidney to other parts of the circulation

To function effectively the kidney must respond to a variety of stimuli One of the most important signals for altering the reabsorption of water by the renal tubule is the antidiuretic hormone Age differences in the inhibition of water diuresis following the intravenous administration of small amounts of pitressin have been observed (Miller and Shock 1953) In these experiments a maximum water diuresis was established by the oral administration of 500 ml water at 6 00 a m , followed by 250 ml water at half hour intervals until completion of the test To ensure maximum urine

of the falling cardiac output. This vasoconstriction is functional in character and can be removed by an appropriate physiological stimulus. Although the tubular epithelium responds to the stimulus of the antidiuretic hormone as quickly in the old as in the young, the functional capacity of the tubular epithelium to perform osmotic work shows a gradual reduction with age.

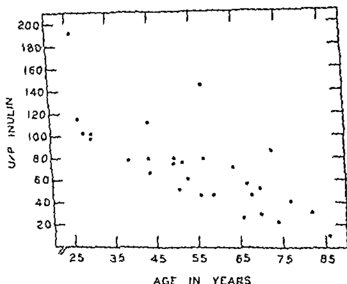


FIG 11 Relationship between maximum U/P inulin following pitressin, and age. The ordinate is the mean U/P ratio for periods 5 and 6. (From Miller and Shock, 1953)

Although these experiments serve to define certain limitations in renal function with increasing age, we must turn to other observations to tell us how effective the aged k

..., there is no evidence of any systematic changes with age. Although Videbaek and Ackermann (1953) reported a slight rise in plasma potassium concentrations, 4.0–4.5 m-equiv/l, between the ages of 25 and 90, the

functional capacities of the tubular cells to perform osmotic work on the glomerular filtrate

The results of these observations lead to the concept that with increasing age, there is a gradual loss of nephrons in the

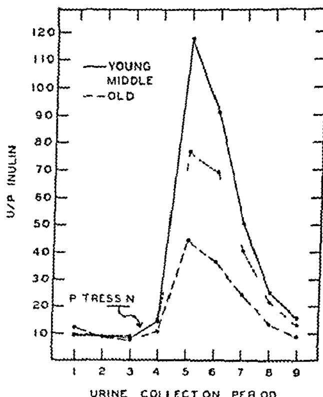
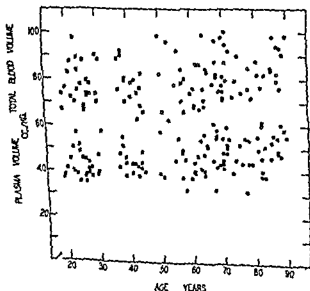


Fig 10 Mean values of U I in il n ratio for each of tl ree age groups before and after tl e intraveno is a l m nistration of pitressin Urine collection periods 1 9 represent n ne consecutive 12 min ite periods Pitress n was administered imme liately after tl e concl ision of period 3

(I rom Miller and Shock 1953)

kidney In addition to these structural losses there are *functional changes* One of these is a gradual increase in the vasoconstriction of the vascular bed of the kidney which further reduces the flow of blood through it, even in the face

Alving (1938) found some evidence that with increasing age there is an accumulation of urea nitrogen in the blood Their data show a slight rise in the fifth decade, but no significant change during the sixth and seventh decades, with a rather sharp increase after the 70th year Most of the total rise from a mean of 12 9 mg urea N/100 ml blood in the 30–40 age



(From Cohn and Shock 1949)

group to a mean of 21 2 mg per cent in the 85–89 year olds occurred after the age of 70 It therefore appears that there some

trend was not statistically significant. The other major electrolytes, sodium and chloride, do not show any age trend (de Billis, 1954; Herbeuval, Cuny and Manciaux, 1954; Lippi and Malerba, 1955). In our own laboratory we have found no

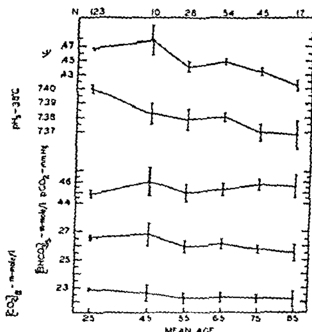


Fig 12 Trends in the acid base equilibrium of the blood of males with increasing age Average curves from top to bottom include percentage of red cells, serum pH at 38°, carbon dioxide tension expressed in millimetres of mercury, serum bicarbonate and blood carbon dioxide content, both expressed in millimoles per litre The vertical lines indicate ± one standard error of the mean. Data for the 25 year determinations taken from Hamilton and Shock (1936)

(From Shock and Yiengst, 1950)

systematic age changes in the total osmotic pressure of the plasma or its water content. The bicarbonate content of the plasma and the pH do not show significant age trends (Shock and Yiengst, 1950). Thus, under basal conditions the kidney is able to regulate the acid-base equilibrium of the body adequately, even to advanced ages (Fig. 12). Lewis and

ammonium chloride produces displacements of the acid base equilibrium in both old and young subjects However, young individuals are able to readjust equilibrium within a period of eight hours, following a single dose of 10 g. of ammonium chloride, whereas the older subjects require as much as 24–36 hours for the process (Shock and Yiengst, 1948) When repeated daily doses of 1 5 m equiv. ammonium chloride/kg. body weight/day were administered to normal subjects for 4–1

wi

(6

load of ammonium chloride (Hilton, Goodbody and Kruesi, 1955) It was also found that the degree of metabolic acidosis induced by a standard dose of ammonium chloride showed a greater severity in the older subjects than in the young. We have now initiated a study of age differences in the ability of the individual to regulate plasma and extracellular fluid

... reduction in discrete renal functions with age, the kidney retains sufficient capacity to regulate both concentrations and volumes fairly closely under conditions of rest However, when experimental displacements are produced, age differences in the speed of readjustment appear

There are obviously many other questions, such as age differences in glomerular permeability and the activity of specific cellular enzymes in the kidney, which remain unanswered

... in the total oxygen uptake for kidney tissue between the ages of 12 and 24 months in the rat, these differences disappear when an appropriate correction for cell number is introduced There are, however, some specific enzymes such as

whi

tion

It is ... these observations to include the

after 12 hours of water deprivation falls from an average of 1·032 at age 20 to 1·024 at age 80–90. Although the absolute magnitude of the decrement is small, it is statistically significant (Lewis and Alving, 1938) and indicates impairment of the concentrating ability of the kidney, which is no doubt a reflection of the reduction in Tm as reported from our studies.

With regard to volume regulation, our observations on a

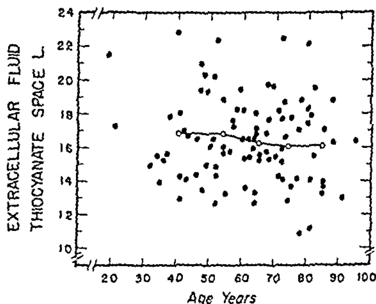


FIG. 14. Relationship between extracellular fluid space (thiocyanate space) and age in males.
(From Shock, 1956)

series of 152 males failed to demonstrate any systematic changes in either plasma volume (Cohn and Shock, 1949) or in total extracellular fluid volume (Shock, Watkin and Yiengst, 1954) as estimated by thiocyanate determinations (Figs. 13 and 14).

Although the aged kidney has a capacity for maintaining acid base equilibrium of the plasma under resting conditions, when an extra load is imposed upon it age differences appear. Thus, for example, we have found that a single dose of

eight hours, following a single dose of 10 g of ammonium chloride, whereas the older subjects require as much as 24–36 hours for the process (Shock and Yiengst, 1948) When repeated daily doses of 1 5 m equiv. ammonium chloride/kg body weight/day were administered to normal subjects for 4–14 days, readjustment of the acid base equilibrium occurred within 5 7 days in the young subjects, but the aged subjects (65–73 years) were unable to attain equilibrium under this load of ammonium chloride (Hilton, Goodbody and Kruesi, 1955) It was also found that the degree of metabolic acidosis

the individual to regulate plasma and extracellular fluid volume following the imposition of an oncotic load.

Thus, the evidence now available indicates that in spite of the reduction in discrete renal functions with age, the kidney retains sufficient capacity to regulate both concentrations and volumes fairly closely under conditions of rest. However, when experimental displacements are produced, age differences in the speed of readjustment appear

There are obviously many other questions, such as age differences in glomerular permeability and the activity of specific cellular enzymes in the kidney, which remain unanswered. Studies on cellular enzymes are now in progress in our laboratory, using the rat as an experimental animal Although we have found a reduction in the total oxygen uptake for kidney tissue between the ages of 12 and 24 months in the rat, these differences disappear when an appropriate correction for cell number is introduced There are, however, some specific enzymes, such as succinoxidase, which show an age reduction which is apparently not dependent on the number of functioning cells in the kidney preparation (Barrows *et al*, 1957). It is our aim to extend these observations to include the

capacity for concentrating specific substances, such as PAH, in tissue slices removed from the kidneys of animals of different ages. It is thus apparent that a great deal of research remains to be done before we can interpret age changes in renal physiology.

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DISCUSSION

Zweymüller: One of the interesting things in your paper, Dr. Shock [illegible] and Tm_G to [illegible]

tubules still able to [illegible]

logical conditions we have a Tm_{PAH} which means that under normal

conditions thus function has an upper ...

tubules that are still present in the old kidney ... N.Y., 77, 686). In other words the able to determine, are just as go ... tressing to me because I am cor ... changes The tubule just cannot ... tomorrow, but unfortunately th ...

Heller ...

body water you wipe ... in renal function ren ... water

Hingerty When y ... cted your subjects, Dr Shock, did you exclude obese patients?

Shock We did not use any index of body weight to exclude patients. I would say immediately that in our population we do not see obese ple over the age of 65 These patients were all males so we do not w anything about the weight of females

Hingerty It seems to me that the decline in kidney function sets in at ut the 50-year mark, and that is about the age when you would expect gher incidence of obesity in the general population

Shock Actually the body surface area decreases with increasing age l groups of subjects we have studied The major factor that contri- s to this reduction in surface area is the body height, which goes n more than body weight There is a wide scatter in height in our lation, but there is a statistically significant linear decrement een the ages of 30 and 90 There is no significant ... ht on age in the population of males ...

...ck Is there any ...

... function studies had no elevation ... this was one of the selection criteria Every

individual in the ren[illegible]
to a specific gravity [illegible]
Alving (1938. *Amer* [illegible]
levels in 100 subjects aged 20 to 80. They found little increment in blood urea up to the age of about 70, but from 70 on it does increase in their data.

[illegible]

that the blood urea did not rise markedly in patients with low protein intake unless the glomerular filtration rate decreased to less than 25–30 ml./min.; in your work the glomerular filtration rate was far above this figure.

[illegible]

point to a diversion of blood from the kidneys due to this insufficient

[illegible]

the cardiac output results that I showed you in the average curve were not determined on the same subjects as the renal functions. We are now measuring cardiac output and renal function in the same subjects simul-

[illegible]

experiments at almost the same time He d d not elevated blood press identical with the vated blood press

Scribner The q posed by these data is is the change in the kidney function, as described, a result of disease in the kidney wearing out with age, or is it simply of the living Heller and ex changeable points?

Shock We done a good many creatinine determinations in balance studies under conditions of a closely regulated diet. However, I have never been able to convince myself that creatinine excretion gives a stable value that is characteristic of the individual, because we have seen some rather wide fluctuations that we have not been able to explain satisfactorily I tried it first with adolescent children and then gave it up as I did not feel it could be determined as a character for the individual But I am exchangeable potassium

Bull The lines you s with age are practically in burns By Probit ar which will produce dea coincidence that you chose your ordinate right scale, but the lines are almost the same in that they take off at just the same age and go down in the same way Burning is a severe stress We have been talking about the elderly having a reduced tolerance to stress and burning is largely a stress affecting water and electrolytes The burn is a convenient measurable lesion, and death occurs with a progressively smaller size of burn with advancing years, which I think probably represents an important aspect of the ageing of a regulation of water and salt

individual in the renal series was able to concentrate his urine at least ... and ... urea ... it in ... se in their data.

that the blood urea did not rise markedly in patients with low protein intake unless the glomerular filtration rate decreased to less than 25–30 ml./min.; in your work the glomerular filtration rate was far above this figure.

In congestive failure or other situations where cardiac output is inadequate, there is usually a decrease in renal blood flow, and an increase in tubular reabsorption of water. The normal concentration test might point to a diversion of blood from the kidneys due to this insufficient cardiac output.

... only not ... facts simul- ... ne ... st ... n ... It seems to me that

... blood urea in a ... showed that in ... urea clearance ... clinical experi- ... per cent without ... l ... r ... t

Borst: Dr. Shock, you can ... rmal kidneys? ... ssure greater than ... lbrich (Olbrich et ... renal functional

Renal growth and regeneration

Both the tentative and the definitive foetal kidneys develop from mesoderm, in intimate relation with the gonads So it is not altogether surprising that the adult kidney resembles the other transient tissues and its life cycle is not completely synchronous with that of the rest of the body (Kennedy, 1957) It would be disastrous to a species, of course, if kidney and body got too far out of step, but any tendency for this to happen during reproductive life would be prevented by natural selection There is some evidence, however, that the kidney atrophies after the climacteric, and in some species such as the rat this may limit life

Most mammals develop their full complement of nephrons soon after birth and postnatal growth of the kidney consists chiefly of lengthening of its tubules, at first by the growth of new cells and later by hypertrophy of existing ones When a rat is about six months old or a man about 30 years, the number of glomeruli in their kidneys begins to decrease, and it may fall to half the young adult value, without pathological change, by eighteen months old in the rat or seventy years in the man (Arataki 1926, Moore, 1931, Roessle and Roulet, 1932) Moore and Hellman (1930) showed that removing one kidney from a rat did not slow down the loss of nephrons from the other so that involution of the kidney is an even more relentless process than that of the ovary, where removal of one gland does delay the loss of oocytes from the other (Mandl and Zuckerman, 1951)

Nowadays chemical analysis can be used to supplement histology in determining the number and size of the cells in a tissue This is because one of the two forms of nucleic acids in cells deoxyribonucleic acid or DNA is confined to the nuclei, as the name suggests it ought to be, while the other, ribo nucleic acid or RNA, is distributed with the bulk of the ordinary protein throughout the cytoplasm So if DNA, RNA and protein are determined at different stages during the growth of a tissue, it is possible to distinguish between

AGE AND RENAL DISEASE

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Introduction

SENESCENCE has sometimes been described as a deterioration in homeostasis Dr Shock showed us that the deterioration may be due to failing renal function, and this reopens an old question of whether the kidney cells themselves are less able to do their work in old people, or whether diseases of the kidney become more frequent with advancing age It seems generally agreed that pathological lesions, particularly of the renal vessels, are very commonly found *post mortem* in old people in whom they were unsuspected during life Oliver (1942) reviewed the controversy as to whether these lesions originate from a primary atrophy of the kidney, or are merely one of the results of generalized arteriosclerosis He decided in favour of arteriosclerosis The other view, that the kidney dies piecemeal, will be re examined here because it seems possible to show that the death of some nephrons leads to pathological changes in the survivors, and some indirect ways in which this may happen will be suggested

One can raise objections to any theory of ageing The major defect of the definition in terms of homeostasis, it seems to the present author, is that the newborn animal finds it just as difficult to maintain a stable internal environment under stress as does the senile one An older definition by Minot (1908), in more structural terms described senescence as the gradual loss by differentiated cells, throughout life, of the ability to grow and to regenerate This idea applies especially well to the kidney, as we shall see

kidneys of the six month old rats. It may be emphasized that these findings accord very well with Minot's definition of ageing As will be shown later, hyperplasia can and does occur in the tubules of older rats, but it does not then represent the normal primary response to loss of moderate amounts of renal tissue, and some additional stimulus, possibly endocrine in nature, is probably involved

Renal Senescence

The compensatory changes that we have been considering are self limiting, and once they have been achieved, the kidney undergoes no further changes for many months. A different sort of tubular change will now be considered In rats killed after 18 months of age [illegible]
in occasional tubules, [illegible]
principally the proxim[illegible]
regular, orderly growth of cells in young rats' kidneys At this age a lot of nephrons have already disappeared, but one would expect the surviving tubules to compensate for their loss by hypertrophy rather than hyperplasia Further, this hyperplasia in ageing kidneys appears to be destructive rather than helpful, because the tubules are often blocked and functionless and eventually become dilated by hyaline casts As age increases still further the kidneys become greatly enlarged and granular in appearance, and microscopically they show chronic interstitial fibrosis, generalized tubular dilatation, and hyaline or fibrotic changes in the glomeruli and smaller vessels These histological changes have been described and illustrated more fully elsewhere (Kennedy, 1951, 1957) The terminal appearance has been studied by numerous pathologists, but since no two agree on a [illegible]

[illegible] and senile nephrosclerosis (Oliver, 1942) have all been used

an increase in nuclei, or hyperplasia, and an increase of cytoplasm, or hypertrophy. This method has shown that the principal increase in the number of nuclei in the kidney of the rat occurs during the first three months of life, *pari passu* with the main growth of the skeleton, and this agrees well with histological findings.

There is a conflict of evidence about regeneration, however. Rollason (1949) showed histologically that mitosis began in the surviving kidney within forty-eight hours of unilateral nephrectomy, whereas Mandel, Mandel and Jacob (1950)

Table I

THE EFFECT OF UNILATERAL NEPHRECTOMY ON THE COMPOSITION OF THE SURVIVING KIDNEY IN RATS AT DIFFERENT AGES

Age at Operation	*Interval before Killing*	*Group*	*Total nitrogen (mg per kidney)*	*RNA phosphorus (mg. per kidney)*	*DNA phosphorus (mg per kidney)*
One Month	Two Weeks	Control (not operated)	11.3	0.273	0.165
		Kidney removed	18.0	0.424	0.233
Three Months	Six Weeks	Control	10.4	0.378	0.181
		Kidney removed	29.4	0.488	0.227
Six Months	Six Weeks	Control	28.1	0.587	0.253
		Kidney removed	41.0	0.717	0.244

were unable to show any increase in kidney DNA even three weeks after the same operation. The difference apparently depends on the age of the animals. Table I illustrates a comparison made by the present author of the effect of unilateral nephrectomy on the composition of the surviving kidney in one-month, three-month and six-month old rats. In the youngest group, which were about the same age as Rollason used, there was a rapid increase in DNA phosphorus. In the middle group the DNA increased less than the RNA and the nitrogen, and more slowly, as Mandel, Mandel and Jacob had found. No hyperplasia at all occurred in the

kidneys of the six-month-old rats. It may be emphasized that these findings accord very well with Minot's definition of ageing. As will be shown later, hyperplasia can and does occur in the tubules of older rats, but it does not then represent the normal primary response to loss of moderate amounts of renal tissue, and some additional stimulus, possibly endocrine in nature, is probably involved.

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The ...

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adiposity and abnormal fat metabolism If the normally fed rats with one kidney were to develop the same pathological renal changes as the overfed rats with two, then it would be reasonable to attribute the lesions to some effect associated

Table II

COMPOSITION OF THE KIDNEYS OF OBESE RATS OR OF RATS WITH ONE KIDNEY REMOVED AT THREE AND SIX WEEKS AFTER OPERATION

Time from operation	*Group*	*Total nitrogen (mg per kidney)*	*RNA phosphorus (mg per kidney)*	*DNA phosphorus (mg per kidney)*
	Control (not operated on)	19 4	0 378	0 183
Three weeks	Kidney removed	27 8	0 504	0 210
	Obese	29 9	0 483	0 227
Six weeks	Kidney removed	29 4	0 516	0 289
	Obese	28 3	0 532	0 303

with overloading They did develop the lesions at the same time as the obese rats at an average age of 15 months Table III illustrates the changes in composition of the kidneys 12 months after carrying out each type of operation on three

Table III

COMPOSITION OF THE KIDNEYS OF OBESE ANIMALS OR OF ANIMALS WITH ONE KIDNEY REMOVED TWELVE MONTHS AFTER OPERATION (OPERATED AT THREE MONTHS OF AGE)

Group	*Total nitrogen (mg per kidney)*	*RNA phosphorus (mg per kidney)*	*DNA phosphorus (mg per kidney)*
Control (not operated on)	34 9	0 749	0 239
Kidney removed	73 5	1 479	0 635
Obese	75 9	1 972	0 785

month old animals Note that in each case the final renal breakdown [illegible]
the period

The pathological renal changes in old rats are almost invariably accompanied by great enlargement of the adrenals frequently by parathyroid hyperplasia, and in the later stages, at least, by cardiac hypertrophy and hypertension Before considering further which is cause and which is effect, a description will be given of a number of ways in which similar renal lesions can be produced in much younger rats in association with the same endocrine and vascular changes

"Senile" changes after renal overloading in younger rats

The first condition in which these lesions were found in fairly young rats was in experimental hypothalamic obesity When the ventromedial part of the hypothalamus is destroyed electrolytically, the appetite of a rat may be doubled for several weeks and the animal becomes grotesquely fat In view of the association of clinical obesity with renal disease and hypertension it is interesting that most of these fat rats developed typical senile kidney lesions about nine months earlier than unoperated controls (Kennedy 1951) If the animals were operated on at three months old they survived nine to 12 months before pathological lesions appeared in the kidneys but the kidneys became enlarged during the period of overfeeding soon after the hypothalamic puncture Moise and Smith (1927) and Addis and Oliver (Oliver 1945) showed that the renal enlargement produced by a high protein diet in rats could eventually cause pathological changes and it seemed possible that this might be the way in which the kidneys were damaged in hypothalamic overfeeding As a first step an examination was made of the chemical changes in the kidneys during the earlier stages of development of the obesity, while the food intake was very high In Table II these are compared with the changes found previously in the surviving kidneys after unilateral nephrectomy and they followed an almost identical pattern This suggested a convenient way to isolate the effect of simple kidney overloading during overfeeding from any possible effect of the subsequent

among the measures that Selye uses, as he says, to "sensitize" the rat to the damaging effect of hormones Nevertheless, we have found that no overgrowth of the kidney occurs in hypophysectomized rats with hypothalamic lesions, although they still have increased appetites and other tissues, such as the liver and gastrointestinal tract, hypertrophy (Kennedy and Parrott, 1958) We also confirmed, as White, Heinbecker and Rolf (1941) first showed, that compensatory growth after partial nephrectomy required the presence of the pituitary However, the late renal changes in our rats were associated with a catabolic rather than an anabolic state of the body as a whole, so it seems unlikely that growth hormone was being secreted in excess

There remains the possibility that adrenal overactivity plays a part in the final renal breakdown Adrenal enlargement and the nephrotic character of the renal defect (Saxton and Kimball 1941) have been mentioned A number of workers have shown that complete or extensive partial nephrectomy is followed by increased urea production (Bondy and Engel 1947 Persike and Addis 1949, Persike, 1950 McCance and Morrison 1956) This has recently been *shown to* be due *to* increased protein catabolism in the liver (Sellers Katz and Marmorston 1957), so it may well be a result of increased adrenal activity Overdosage with adrenal steroids can certainly cause renal breakdown assoc[illegible]

[illegible]) associated with such experiments We have learned little from the serum electrolytes of our rats because any changes that *might implicate* the adrenal are obscured by the general electrolyte retention of incipient uraemia Morrison and Gordon (1957) however, have shown that increased urea excretion *during* starvation occurs both in partially nephrectomized and senile rats *before* obvious renal damage and is accompan*ied by an* increased potassium loss

Another renoprival effect that may hasten the end of the kidney is hypertension, although again the exact relation

animal at operation, and in fact the age at which the final breakdown occurred was almost constant To take the extreme case, rats over a year old frequently failed to establish any new renal equilibrium after either type of overloading, but rapidly developed pathological lesions

The age at which renal failure occurred was advanced still further by increasing the renal loading, either by a more extensive partial nephrectomy, or by combining unilateral nephrectomy with overfeeding It is sometimes said that different species tolerate the removal of different proportions of their renal tissue It is difficult to see how a valid comparison can be made when the critical amount of kidney depends so much on the age of the animal We found that weanling rats recovered and survived for many months after losing five sixths of their kidneys, while nine month old adults often developed acute tubular necrosis after the same operation A probable explanation for the latent period in the younger animals is that it represents the time for the further loss of nephrons due to ageing to reduce the available kidney below the critical level It remains to consider the part played by the associated metabolic and endocrine disturbance in destroying the kidney

Endocrine stimuli to renal hyperplasia

A number of hormones are renotrophic They include growth hormone (White Heinbecker and Rolff, 1949) thyroid hormone (Korenchevsky and Hall, 1944) and testosterone (Korenchevsky and Ross 1940) The results of treatment with growth hormone are particularly suggestive Acute overdosage can lead to rapid kidney destruction but treatment of a young rat for only a few days apparently causing no damage at the time can lead to the appearance of pathological lesions months later (Selye 1951) From the limited descriptions and photographs available no difference can be seen between these and the spontaneous lesions of older rats or those which develop after partial nephrectomy Interpretation is complicated because partial nephrectomy is

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suggested that the loss of renal tissue in excess of a critical amount leads to additional renotrophic stimuli, probably related to overactivity of the adrenal cortex and to hypertension, which hasten the end of the remaining nephrons.

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between cause and effect is uncertain Wilson and Byrom (1939, 1941) showed that the production of hypertension by "clipping" one kidney could lead after a prolonged latent period to pathological lesions in the other kidney They attributed these lesions to hypertension, because their development seemed to be arrested and the hypertension cured by removing the ischaemic kidney Goldblatt (1947) pointed out that all the lesions Wilson and Byrom had described could occur spontaneously in rats without hypertension More recent work, reviewed by Floyer (1957), suggests that removal of the clip, so restoring some of the lost excretory function, is a much better protective measure than removing the ischaemic kidney, which frequently increases the hypertension The importance of extrarenal or renoprival factors in producing permanent hypertension now seems well established and certainly fits with our experience, and apparently with Goldblatt's, that hypertension and vascular changes are a late feature of the spontaneous renal disease of rats

Much remains to be done, but it is hoped that some progress has been made towards establishing the thesis, stated at the beginning of this paper, that the essential vicious cycle of renal disease in old age, in one species at least, is the destruction of surviving nephrons by overloading after the normal renal atrophy of old age has reached a critical stage

Summary

The kidney of the rat and of most mammals including man, begins to atrophy while the animal is still young Pathological changes in the kidney become more frequent during involution Irregular and apparently purposeless hyperplasia of tubular cells is a prominent feature of such lesions Hyperplasia occurs in the tubules of growing rats both as part of normal development and as a response to a moderate increase in the excretory load, *but it is not normally seen after the main* growth of the skeleton is completed The stimulus to normal renal growth probably arises in the pituitary gland It is

(less than 20 g daily) I have no comparison with …
who continued eating normal …
that our patients c…
nausea usually disa…
are also in better c…
protein intake often … and sometimes in
a slight rise in seru… poor diet does not
prevent a grad… However this decline
is usually slo… years of useful life
A high diasto… result is a very bad prognostic factor As long
as we have no control group we cannot produce convincing evidence
that an untreated patient will not live as long as our 'maltreated'
patients

Kennedy Have you done any liver function tests in a situation where serum albumin is falling in spite of a high protein intake, Prof Borst? There may be a possible connexion with the increased liver protein breakdown when one removes the kidney (Sellers, Katz and Marmorston, (1957) *Amer J Physiol*, 191, 345)

Borst No liver function tests were done, and we only have data on the serum proteins There is no increased γ globulin as is usually found in chronic hepato-cellular disease We had, however, some evidence of a deleterious effect of the low protein diet More cases of tuberculosis were seen than would be expected in similar patients on a normal protein diet and two patients died from miliary tuberculosis Probably the extremely low protein diet reduces the resistance against the tubercle bacillus in spite of the fact that the patients do not lose weight

Talbot How do you define a low protein diet?

Borst It is less than 20 g /day To control the diet and determine whether or not the patient adheres to it, 24 hour urine portions are regularly examined for nitrogen excretion We also determine creatinine excretion to be sure that urine collection is complete The 24-hour creatinine output is very constant This output is determined for every kidney patient during clinical observation, and we use the figures for comparison with the nitrogen output when the patients are under control in the out patient department Many adhere to the diet and go along very well for several years

Fejfar We have had similar experiences in Czechoslovakia This treatment originated in the experiments of Thomas Addis (1948 Glomerulonephritis Diagnosis and Treatment New York Macmillan), who showed that partially nephrectomized rats kept on a higher protein intake could not survive as long as the animals with a low protein diet We therefore started to use a low protein diet in all patients with chronic glomerulonephritis Usually we give 0 5–0 7 g /kg body weight per day in the diet (but no less than 0 5 g /kg) plus the amount lost in the urine Of course, children and those with the nephrotic syndrome are given larger amounts of protein It is very difficult to judge long term results as we have no control group for this treatment Nevertheless we do think we can prolong the life of patients with chronic nephritis on this low protein diet

WHITE, H L, HEINBECKER P, and ROLF, D (1949) *Amer J Physiol.*, 157, 47
WILENS S L and SPROUL E E (1938) *Amer. J Path*, 14, 201
WILSON C, and BYROM F B (1939) *Lancet* 1, 136
WILSON, C, and BYROM, F B (1941) *Quart J Med* 10, 65

DISCUSSION

Sayer You said that this renal damage in the obese rat might be a question of protein overloading Did you try feeding these rats on an *isocaloric diet but with half the protein content?*

Kennedy I have tried it as a short term experiment but I did not carry it to its logical conclusion There was no renotrophic effect

Sayer Over feeding is itself a stressful activity in the Selyeian sense and that alone might lead to adrenal over activity Certainly there is clinical evidence that it may Obese people who give evidence of increased adrenal steroid production may cease to do so after they have been put on a diet and have had their weight brought down to normal

Kennedy To answer that I must challenge the question of whether in fact stress ever produces renal lesions in the rat I can do that quickly by quoting some recent work by Crane Baker and Ingle (1958 *Endocrinology* 62, 216 and Crane and Ingle *Endocrinology* 62, 174) who have studied a large number of so called stresses which sound quite barbaric and have found that the only one which produces what Selye calls the stressed kidney is exposure to cold Selye has always said that this is the most effective and these workers now say that it is the only effective stress Under those circumstances the rats eat twice as much food If they are then fed isocalorically as you suggest with a high caloric diet made up with carbohydrate and fat they do not develop lesions These workers attribute renal lesions to overloading with salt I choose protein

Talbot Will you take this as evidence in favour of restricting the protein intake of patients with handicapped renal function?

Kennedy I can see that it would be a dangerous thing to press a trophic stimulus like a high protein intake too far in an attempt to get recovery Are you thinking of chronic renal disease or a recovery from acute damage?

Talbot Both

Kennedy Purely from my own findings I would have said that I could see no point in producing additional renal growth in trying to help recovery of the kidney by giving a high protein diet if the object was simply to replace protein lost from the body then my results of course are not relevant I think the problem of a high protein intake has to be studied from this point of view on the human and we cannot answer from the work on the rat Moreover there may be a totally different limitation to the structural renal reserve in the rat which has a kidney of completely different anatomical character

Borst We treat all patients with a kidney function of less than 10 per cent of normal with a diet adequate in calories but very poor in protein

(less than 20 g daily). I have no comp...

... patients ... pression

... also in better condition We hav... often gain weight, and in other respects ... Their prote...

Kennedy ... long as our 'maltreated' ... seru... Ther... ha...

... or not the patient ... g day. To control the diet and determine regularly examined for nitrogen excretion. 24 hour urine portions are excretion to be sure that urine collection is complete. We also determine creatinine creatinine output is very constant. This output is determined for every kidney patient during clinical observation, and we use the figures for comparison with the nitrogen output when the patients are under control in the out patient department. Many adhere to the diet and go along very well for several years.

Fejfar We have had similar experiences in Czechoslovakia. This treatment originated in the experiments of Thomas Addis (1948. Glomerulonephritis Diagnosis and Treatment New York. Macmillan), who showed that partially nephrectomized rats kept on a higher protein intake could not survive as long as the animals with a low protein diet. We therefore started to use a low protein diet in all patients with chronic glomerulonephritis. Usually we give 0.5–0.7 g/kg body weight per day in the diet (but no less than 0.5 g/kg), plus the amount lost in the urine. Of course, children and those with the nephrotic syndrome are given larger amounts of protein. It is very difficult to judge long term results as we have no control group for this treatment. Nevertheless we do think we can prolong the life of patients with chronic nephritis on this low ...

Richet: In populations that are said to eat a lot of proteins, for instance Eskimos, what is the state of the kidney? Do such people often die from chronic nephritis? They are generally said to eat 5,000 cal./day, mostly fat and proteins.

a man of about 40–45.

Dr. Talbot, you mentioned the amount of protein given in cases of chronic nephritis. In Paris we put some chronic nephritic patients on an

they would never live anyway; we prefer to have a patient with perhaps a shorter life, but healthy, than the other way round.

Fourman: Dr. Kennedy, why did you imply a relationship between catabolic reactions, adrenal hyperplasia and Selye's results with cortexone acetate?

Kennedy: The catabolism would require over-secretion of Compound F, of course. However, Hechter and Pincus (1954. *Physiol. Rev.*, 34, 459)

results with cortexone acetate?

Kennedy: Yes, in that cortexone acetate was the particular steroid

tassium depletion are similar in

repeated these experiments with dietary potassium depletion and cor
young rats and were unable
conclusively at Cambridge.
to permanent damage from
h Dr. Fourman's suggestions
lye.

Kennedy Dr Fourman and I have looked at potassium-deficient

that

be worse in older rats

Kennedy. It is a vicious cycle and we are coming into it at different points

RENAL FUNCTION IN RESPIRATORY FAILURE

D A K Black

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With increasing age, the functional capacity of the lungs and of the kidneys declines Respiration is embarrassed by increasing rigidity of the chest wall, and there is also an increase in the respiratory dead space of the lung itself in older subjects (Comroe *et al*, 1955) The kidneys lose efficiency in consequence of a progressive loss of nephrons, which may reduce the nephron population to 60 per cent of the original number, the impairment of renal function is indicated by a fall in the clearance of inulin and of *p* aminohippurate, and in the maximal reabsorptive capacity for glucose (Tm_G) (Shock, 1952) The blood pH in old people is a little lower, and their plasma returns more slowly to its previous level after imposed loads of either acid or alkali These various encroachments on functional reserve are probably of no great moment in healthy old folk leading a normal life, but they are brought into prominence when respiratory function is pathologically impaired by the related changes of chronic bronchitis, bronchospasm, and emphysema In an urban population, the incidence of chronic bronchitis in old people has been found to be 40 per cent (Sheldon, 1948), this common illness leads in time to gross respiratory failure, with the patient afflicted by anoxia, hypercapnia, and increased pulmonary vascular resistance in varying degrees There are several ways in which advanced respiratory failure can increase the demands on the kidneys, and also diminish their functional capacity This communication outlines the effects on renal function of chronic hypercapnia and of cardiac failure secondary to emphysema (cor pulmonale)

Hypercapnia The effects of acute hypercapnia, usually induced by inhalation of 5–10 per cent CO_2, have been reviewed by Pitts (1953) There is a fall in plasma pH and a rise in pCO_2 the urine formed is acid, and the reabsorption of filtered bicarbonate is virtually complete, although the amount of filtered bicarbonate has been increased by the experimental procedure Enhancement of bicarbonate reabsorption is the most striking change in renal performance induced by acute hypercapnia and it persists when the fall in plasma pH is prevented by infusion of bicarbonate, so that in this context rise in pCO_2 seems to be the more relevant stimulus to bicarbonate reabsorption The reabsorption of bicarbonate is also increased in subjects depleted of potassium, in whom intracellular pH is probably decreased, so it seems quite likely that the effect of raised pCO_2 on bicarbonate reabsorption is mediated by a fall in the pH of the renal tubule cells Apart from this rather striking change in bicarbonate excretion the output of electrolytes is not significantly affected by short periods of hypercapnia, although there is a transient water diuresis (Barbour *et al*, 1953)

It is not clear how far the information obtained from studies of acute hypercapnia can be applied to the situation of chronic hypercapnia found in emphysematous patients Here a steady state has been established at a new level of plasma pH and bicarbonate concentration The electrolyte composition of plasma and red cells in emphysemato[illegible]
pat[illegible]
pe[illegible]
ac[illegible] [illegible]ation (Platts and Greaves 1957) For
exampl[illegible]
patient[illegible]
lower t[illegible] [illegible]atory acidosis

There are few observations on the renal response to chronic respiratory acidosis in man As part of a study on the effect of Diamox Nadell (1953) reports observations on 24 hour specimens of urine from two patients with respiratory acidosis

The mean urinary pH in these two patients was 6 26 and 6 67, no more acid than specimens from two 'controls' with mean pH of 6 18 and 6 20 The mean excretions of bicarbonate were 7 5 and 15 2 m mole/day, compared with 10 1 and 4 8 m mole/day in controls Ammonium excretion was some what higher, and titratable acidity somewhat lower in the patients with respiratory acidosis than in the controls, and it has been reported that renal glutaminase is increased in experimental respiratory acidosis There were no striking differences in 24 hour output of sodium, potassium or chloride These findings are consistent with the view that renal adaptation has included increased synthesis of ammonia allowing the excretion of hydrion at a higher urine pH than in acute respiratory acidosis, without increase in urinary buffer (the excretion of phosphate was lower than in the controls)

In preliminary observations on four patients with respiratory acidosis, my colleague Dr J Timoner has found a pH range in urine of 5 1 to 6 7, with ammonium excretion up to 65 μ equiv /min and titratable acidity up to 60 μ equiv /min After a standard load of ammonium chloride (0 1 g /kg body weight), two patients excreted 76 5 and 81 3 μ equiv of ammonia, and 26 3 and 46 2 μ equiv of titratable acid per minute The ammonium excretion is just above the normal range found by Davies and Wrong (1957) These two patients were aged 57 and 60, and seem to have retained the capacity of the renal tubule cells to form ammonia in response to an acid stimulus

Renal function in cor pulmonale In the cardiac failure associated with emphysema the cardiac output is commonly increased, and the patient has warm extremities Terminally, the limbs become cold, the blood pressure falls and the cardiac output at this stage is reduced Davies and Kilpatrick (1951) showed that even in the high output phase of cor pulmonale the circulation through the kidneys and the glomerular filtration rate were substantially diminished These findings have been confirmed by Lewis and his co

workers (1952) A moderate degree of urea retention, presumably on the basis of relative renal ischaemia, is common in cor pulmonale (Simpson, 1957), as in other forms of heart failure In patients dying from heart failure, the output of urine may be reduced to below 500 ml /day, but complete suppression of urine does not seem to have been recorded, even in the terminal stages It is perhaps of some interest, therefore, that over the past ten years we have seen two patients, both with cor pulmonale, who became anuric (Black and Stanbury, 1958) One of them, a girl of 20 with widespread bronchiectasis and a terminal bronchopneumonia, had an eight day period of extreme oliguria, during which her blood urea rose to 158 mg /100 ml She was treated conservatively, urine was again formed, and the blood urea fell to 76 mg /100 ml She continued to pass considerable amounts of dilute urine until her death a week after the end of the anuric period The second patient, a man of 44, passed no urine for over 24 hours, and had no urine in his bladder after death Both these patients had hypotension and cold extremities, and were presumably in the low output phase of cor pulmonale, but cardiac output could not of course be measured Both of them had central cyanosis, but only the second had a raised pCO_2 in the plasma The main factor in causing anuria was probably renal ischaemia, but this may have been aggravated by arterial desaturation

Both these patients had hyperkalaemia and low plasma sodium This association is fairly common in patients with acute renal f

partial replacement by sodium

These observations in patients with terminal cor pulmonale are possibly of little more than academic interest, but they perhaps constitute yet another argument for the early treatment of intercurrent infections in patients with

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output failure

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DISCUSSION

Milne: I am not convinced, Dr Black, that the anuria you mentioned [illegible] ...sion of chronic respiratory [illegible] dary staphylococcal pneumonia. A severe respiratory infection of itself in some cases seems to be able to precipitate anuria, and I myself prefer to relate your experience to infection rather than to the biochemical changes of chronic respiratory acidosis.

[illegible] a tremendous respect for [illegible] to think we should avoid [illegible]. To the chemist bicar- [illegible] is a base itself. Bicar- [illegible] do not [illegible]

When you say infection, do you mean infection leading ... fall in [illegible]

Milne: No, [illegible] ing is that these cases occurred in young adults without any evidence

whatsoever of chronic respiratory disease, and that a severe respiratory infection, for some reason that I do not know, may cause acute tubular necrosis, for which there is autopsy proof in one case.

Black This would really bring it into the whole group of peripheral circulatory changes

McCance This seems to me a matter which is wide open to experi-

Borst We have just had an autopsy on a very obese patient who died with bilateral cortical necrosis I am ashamed to say that she had been under-examined As in Dr. Black's cases she was admitted with a respiratory infection which was treated with penicillin, and in a few days the infection was under control She was up and about until we dis-

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Hanley (1957 Chronic Bronchitis, Emphysema, and

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had one patient with a

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DISCUSSION

Milne I am not convinced Dr Black that the anuria you mentioned in your two cases is in any way related to the chronic respiratory disease During the last influenza epidemic in this country some cases of anuria were associated with influenza I know of one case in Dundee and we ourselves have personally studied three cases Two of those we saw recovered and one died The one that died showed typical acute tubular necrosis, the other two showed a clinical course typical of tubular necrosis None of these patients gave any sign of chronic respiratory

dary staphylococcal pneumonia A severe respiratory infection of itself in some cases seems to be able to precipitate anuria and I myself prefer to relate your experience to infection rather than to the biochemical changes of chronic respiratory acidosis

do not

fall in

cardiac output and renal vasoconstriction or do you mean an infection of the kidney?

Milne No certainly not an infection of the kidney All I am stressing is that these cases occurred in young adults without any evidence

WATER AND ELECTROLYTE METABOLISM IN CONGESTIVE FAILURE

Z FEJFAR

Institute for Cardiovascular Research, Prague—Krč

The rôle of the kidney in congestive failure

THE genesis of abnormal water and electrolyte metabolism in congestive failure is at present generally attributed to impaired renal function It was previously thought that increased systemic venous pressure (and hence the imbalance of Starling forces in the capillaries) was the main factor initiating these phenomena Warren and Stead (1944) observed in some cardiac patients an increase in body weight after the administration of salt before any significant rise in central venous pressure This indicated that another mechanism might be responsible for the retention of salt and water in chronic congestive failure Merrill (1946) confirmed the earlier findings of Seymour and co workers (1942) that patients with congestive failure have a diminished renal blood flow, moreover he found that the decrease in renal blood flow was far greater than the diminution of cardiac output

It was however, not clear whether the retention of electro lytes and water in chronic congestive heart failure was due to a primary decrease in renal function or to the decrease in renal blood flow and function as a consequence of the increase in central venous pressure

It appeared to us in 1947 (see Brod and Fejfar, 1949, 1950) that only observations of haemodynamic events at the time when water balance was changing could elucidate this problem Patients with heart disease on the borderline of right heart failure usually have a low urine output during the day, but an increased urine flow at night This spontaneous diuresis

attempt to compensate them artificially by getting their serum bicarbonate levels up. We treated a 50 year old man with acute respiratory acidosis whose initial bicarbonate figure was 40 m-equiv/l and the blood pH breathing room air about 7.28. When he went into oxygen he became unconscious rather quickly, presumably due to the decrease in ventilation from the relief of anoxia. He was removed from oxygen and over the next 18 hours dialysed through a cellophan bag in his stomach using a fluid containing 50 m-equiv/l sodium bicarbonate in 5 per cent glucose. The dialysis elevated his serum bicarbonate to 64 m-equiv/l despite a negative sodium balance of 200 m-equiv. The sodium was lost mainly in the urine. The high serum bicarbonate elevated his blood pH breathing room air to 7.55. When he again went into oxygen his blood pH fell to 7.45 and he did not become unconscious. His anoxia disappeared despite the fact that his ventilatory rate slowed from 9 litres per minute to 3 litres per minute. [illegible] hours his kidneys sustained his ser[illegible] [illegible]/l by excreting [illegible]

E[illegible] [illegible]arcosis' is actu[illegible] [illegible] high pCO_2. The results also sugg[illegible] [illegible] serum bicarbonate renal compensation for the [illegible]atory acidosis may be incomplete in this acute situation. Gastrodialysis makes it possible to treat the acidosis without resorting to sodium administration, which is contraindicated because of the heart failure from cor pulmonale.

decompensation, the central venous pressure being normal (Fejfar and Brod, 1949; Blegen and Aas, 1950; Werko *et al.*, 1952*a*; Himbert *et al.*, 1954; Werko *et al.*, 1955).

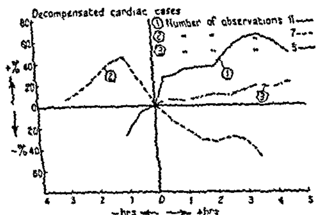


Fig 2 presents the individual values of renal blood flow in normal subjects and in patients with heart diseases. All patients are divided into five groups according to the clinical degree of heart failure

In the first group are clinically compensated patients. The second group includes patients with a slight to moderate dyspnoea on effort, in the third are those with marked dyspnoea on effort, orthopnoea or attacks of nocturnal dyspnoea and acute pulmonary oedema. The fourth group covers patients with signs of right-sided decompensation who responded well

reflects a temporary improvement of the impaired water balance It runs its course within a few hours It was there fore possible to follow the sequence of events and investigate the relationship between central venous pressure, systemic and renal haemodynamic changes, and renal function

Cardiac output, right auricular pressure, water content of plasma, and renal function (renal blood flow, glomerular filtration rate and excretion of electrolytes) were studied from the early hours of the afternoon until the following morning in ten normal subjects and 25 patients with heart disease of different origin 19 of them having congestive failure of varying degree (Brod and Fejfar 1949, 1950, Fejfar and Brod, 1950*a b d*)

Cardiac output was measured by a direct Fick method and right auricular pressure by a water manometer attached to the cardiac catheter changes of water content in plasma were assessed from the percentage change in plasma proteins, haematocrit and the disappearance curve of Evans blue Renal plasma flow was estimated by the clearance of PAH (*p aminohippuric acid*) *glomerular filtration rate by the clear-ance of inulin*, and chlorides by the Van Slyke and Hiller (1947) modification of Sendroy s method

A nocturnal diuresis was observed in 11 patients with con gestive failure In none of them was it preceded by a decrease in right auricular pressure On the other hand the increase in urine output at night started in all these patients with an elevation *in renal blood flow* *The decrease in urine flow at* night occurred in seven decompensated cardiacs in all of them it was associated with a diminution in renal blood flow (Fig 1) The increase in renal blood flow was not related to a similar change in cardiac output which increased simultane ously in only half of the investigated subjects

There is thus evidence in dynamic observations that the increase *in central venous pressure in congestive failure* is not the primary cause of cardiac oedema the main factor being impaired renal function

A low renal blood flow with a diminished glomerular

filtration rate and increased tubular reabsorption of electrolytes was also found in patients with left-sided failure and with mitral stenosis without any clinical evidence of right-sided decompensation, the central venous pressure being normal (Fejfar and Brod, 1949; Blegen and Aas, 1950; Werkö *et al.*, 1952a; Humbert *et al.*, 1954; Werkö *et al.*, 1955).

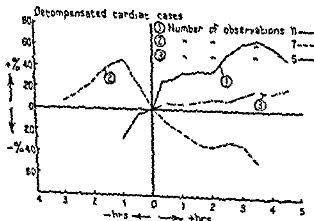


FIG 1 Composite diagram showing percentage changes ...

Fig. 2 presents the individual values of renal blood flow in normal subjects and in patients with heart diseases. All patients are divided into five groups according to the clinical degree of heart failure.

In the first group are clinically compensated patients. The second group includes patients with a slight to moderate ... on e ... acut... The fourth group covers patients with signs of right sided decompensation who responded well

to digitalis, and in the fifth group are patients *refractory to* the usual methods of treatment

It may be seen that patients without right sided failure have a decreased renal blood flow in comparison with the values in normal control subjects

On the other hand increase of pressure in the renal vein brought about by a partial occlusion (Selkurt, Hall and

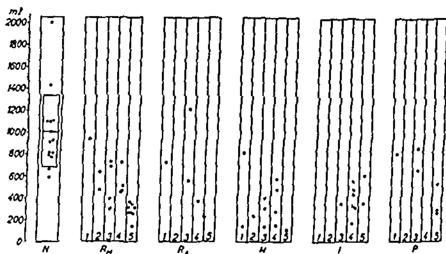


Fig 2 Renal blood flow in normal sub… (R_H and R_{Ac}) hypertensive (H) isch… disease All patients are divided into … degree of heart failure

Spencer, 1949), or by an increased abdominal pressure (Bradley and Bradley, 1947), is followed by only a small diminution of the renal blood flow

Maxwell, Breed and Schwartz (1950) measured pressure in the inferior vena cava in 17 healthy subjects and ten patients with congestive failure The mean pressure in healthy subjects was 15 2 cm H_2O, and in patients with congestive failure 27 cm H_2O From the measured values of pressure they calculated that the increase of renal resistance due to the elevation of pressure in renal veins would reduce renal blood flow by about 14 per cent. *The actual decrease in renal blood* flow in congestive failure is far greater (see Fig 2)

Farber and co workers (1951, 1953) studied in man the effect of an increase of pressure in the vena cava produced by means of a balloon above and below the orifice of the renal veins In both procedures there was a diminution of renal blood flow, glomerular filtration rate and excretion of water and electrolytes

The increased central venous pressure in congestive failure may, of course, contribute to reduction in renal function (Briggs *et al*, 1948, Bradley and Blake, 1949, Earle *et al*, 1949) It determines the distribution of retained water and electrolytes which in left sided failure is in the lungs and in congestive failure mainly in the lower part of the body.

The nature of renal changes in congestive failure

The nocturnal increase of diuresis and renal blood flow in our investigated patients with congestive failure was also associated with an elevation of glomerular filtration rate and with a decrease in tubular reabsorption of water and electro lytes This may be seen in Fig 8, which covers 20 spon taneous changes in urine flow in 14 patients with congestive failure The lower urine output was always taken as the initial value (100 per cent)

The ...

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(R

la' ... on the average by 55 5 per cent (from 6 to 146 per cent) Only three times was the increase in renal blood flow smaller than 20 per cent In 14 subjects in whom it was measured cardiac output (CO) rose significantly in six instances fell in three and did not change in five It is clear that the increase in renal blood flow could not depend on the primary increase in CO This is confirmed by an increase in the renal fraction of cardiac output in all instances except one, in ...

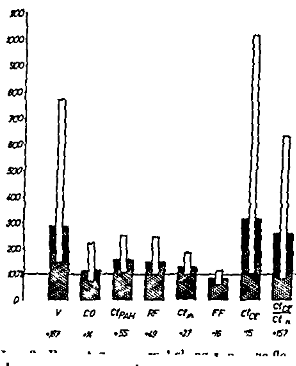
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was 27 ... per cent, with the range − 4 5 to + 82 per cent.

The elevation of renal blood flow was effected in the great majority by a decrease in postglomerular resistance, the filtration fraction diminishing 17 times and increasing in only three instances

The increase in chloride clearance was of the same order as



clearance to glomerular filtration rate $\left(\frac{}{Cl_{In}}\right)$ The mean percentage change (▧) and range (□) in 14 patients with heart failure are presented The lower urine output was taken as 100 %

the elevation of urine volume The mean increase was 215 per cent, range 2 to 910 per cent *The ratio of chloride clearance to glomerular filtration rate rose on an average by* 157 per cent (range −17 6 to +530 per cent)

According to Wesson, Anslow and Smith (1948) some 85 per cent of the filtered sodium and chloride is reabsorbed by an active mechanism in the proximal tubule, irrespective of the amount filtered The reabsorption of the remaining 15 per cent of sodium and chloride is limited by a fixed maximal rate at which the distal tubular cells are able to reabsorb these electrolytes Whenever the tubular chloride load decreases with a fall in glomerular filtration rate in the presence of this maximal reabsorption capacity, almost all of the filtered chloride is reabsorbed Merrill (1949), Mokotoff, Ross and Leiter (1948), Selkurt, Hall and Spencer (1949), Stead (1951) and others are of the opinion that in congestive failure this mechanism leads to the maximum reabsorption of electrolytes and water, that is to say that the diminution of glomerular filtration is such that with a normal unchanged tubular reabsorption, water and electrolytes are retained

Our results are not in accord with the hypothesis of Wesson, Anslow and Smith In patients with severe congestive failure, glomerular filtration rate did not rise towards normal levels at the time of nocturnal diuresis, in spite of this, the amount of excreted chloride was far greater than the quantity of chloride excreted at night in healthy subjects with a normal glomerular filtration rate Fig 4 demonstrates that the tubular reabsorption of chloride can vary markedly with a constant tubular chloride load It is clear, of course, that at a given chloride load less chloride is reabsorbed at a high than at a low urine flow

The concentration of chloride in urine exceeded its plasma level in only seven out of 24 observations at high urine flow The increased urine flow, therefore, cannot be explained on osmotic grounds by an increased excretion of chloride

The lower elimination of electrolytes and water [illegible]

[illegible] as well The same conclusion is stated by Briggs and co workers (1948), Kattus and co workers (1948) Davis and Shock (1949), Newman (1949),

Humbert and co workers (1954), Cort (1955*b*), Cort and Fencl (1957), and others

Doyle and Merrill (1957) studied renal function in 18 patients with congestive failure in a supine position and tilted in a passive erect posture The changes were qualitatively similar to those in normal subjects There was a further depression of renal plasma flow, glomerular filtration rate and also a decreased urine flow and a fall in the excretion of the

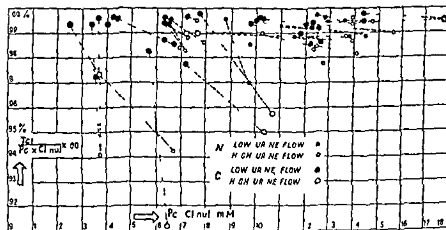


FIG 4 Relationship of the amount of the chloride filtered ($P_{Cl} \times Cl_{inul}$) and reabsorbed $\left(\frac{T_{Cl}}{P_{Cl} \times Cl_{inul}} \times 100\right)$ in individual subjects at high and low urine flows Values in individual subjects are connected with dotted lines *N*—normal control subjects *C*—patients with heart disease See text for details

electrolytes In accord with our previous findings, with nocturia the decreased urine flow in the erect posture was closely correlated with changes in renal plasma flow There was, on the other hand, a very poor correlation between changes in glomerular filtration rate and sodium excretion

In these observations there was an indirect relationship between the tubular reabsorption of electrolytes and water and renal blood flow The tubular reabsorption increased when renal blood flow fell and *vice versa*

This finding does not characterize congestive failure alone

Bucht and co-workers (1953) studied the haemodynamic changes together with the excretion of sodium in eight healthy human subjects during muscular exercise of varying degree As long as the effort was small (oxygen consumption not above 500 ml /min), an increase of CO was found without significant effect on renal blood flow, glomerular filtration rate or excretion of sodium A greater muscular effort (oxygen consumption about 1,000 ml /min) was characterized by a marked increase in CO (almost double) and a simultaneous fall in renal blood flow and the renal fraction of CO The excretion of sodium and water fell Glomerular filtration rate and pressure in renal veins did not change significantly Similar results were observed in patients with heart disease (Judson *et al* 1955, Himbert, Scébat and Théard 1956) Increase of tubular reabsorption was therefore responsible for the diminished excretion of sodium and water

The close relationship between renal blood flow and excretion of electrolytes in congestive failure is striking We have expressed the opinion (Brod and Fejfar, 1950) that decreased renal blood flow directly impairs the excretion of water and electrolytes A smaller glomerular filtration rate diminishes tubular electrolyte load and, owing to a slower flow of tubular urine a greater proportion of the filtered amount is reabsorbed We could not of course, exclude another possibility that increased reabsorption of water and electrolytes in the renal tubules could occur parallel with, but independently of the diminished renal plasma flow, i e the stimulus for the renal vasoconstriction could directly influence the function of renal tubules, leading to an increased reabsorption of salt and water.

Humoral and neural regulatory mechanisms in congestive failure

Some known humoral and neural factors can alter the function of renal tubules In the urine of patients with congestive failure renin (Merrill, Morrison and Brannon, 1946), VEM (vaso excitor material) and VDM (vasodepressor

material) have been found (Edelman *et al*, 1950) Extracts of urine from patients with congestive failure contain anti diuretic materal (Bercu, Rokaw and Massie, 1949, 1950) with a great sodium retaining activity (Deming and Luetscher, 1950*a*,*b*), which disappears when the patients become compensated (Luetscher, Deming and Johnson, 1950, 1951) The substance responsible for this is aldosterone (Luetscher and Johnson, 1954) An increased excretion of aldosterone is not characteristic only of congestive failure, but accompanies nephrotic and cirrhotic oedema as well A permanent increase of aldosterone under these conditions is called secondary aldosteronism (Conn, 1955 Bartter, 1956, Milne and Muehrcke, 1956, Thorn *et al*, 1956, Liddle, Duncan and Bartter, 1956, Wolff, Koczorek and Buchborn 1957)

The increased secretion of aldosterone in congestive failure may be important in some patients, as can be seen from the favourable effect of bilateral adrenalectomy (Thorn *et al*, 1956)

Buchborn (1956) estimated the activity of plasma anti diuretic hormone (ADH) by a sensitive biological method on the toad, together with serum osmolarity He found a close indirect correlation between the plasma ADH and serum osmolarity in 14 normal subjects, in patients with hepatic cirrhosis, in compensated cardiac patients, and also in patients with congestive failure The increased plasma level of ADH in congestive failure is not therefore primary, being an expression of the homeostatic function of ADH, regulating osmotic pressure in the organism (Buchborn, 1956)

Neither ADH nor aldosterone significantly influences circulation in the kidneys Their main effect is on renal tubules where they increase the reabsorption of water (ADH) or sodium (aldosterone) In addition we have already indicated that the vasoconstriction in the kidneys, together with diminished elimination of sodium, occurs during a short muscular effort (10 minutes, Bucht *et al*, 1953) The effect of aldosterone would be slower According to Bartter (1956) the excretion of sodium in a patient with Addison's disease did

not start to fall until more than an hour after intravenous injection of 40 μg aldosterone

It would appear to us, therefore, that neither of these humoral substances is the primary cause of the retention of salt and water in heart failure

The results of haemodynamic changes in human subjects following intravenous injection of Dibenamine called our attention to the importance of reflex (neurohumoral) regulation in the genesis of haemodynamic changes in congestive failure

Blockade of adrenergic impulses by Dibenamine in patients with heart failure caused a diminution of a high peripheral vascular resistance and central venous pressure Cardiac output increased Renal blood flow rose in a great majority of investigated subjects, suggesting that this was independent of the increase in CO These changes were not produced by blocking the adrenergic impulses in the heart or by an increased secretion of adrenaline (Fejfar and Brod, 1950c, 1951, 1954, Brod Fejfar and Fejfarová 1951, 1954) (Fig 5) The increase in renal blood flow in seven out of nine patients in congestive failure was accompanied by a rise in urine flow and an increased elimination of sodium or chloride

We were able to conclude from our results that, with the *onset of congestive* failure, reflex (neurohumoral) vasoconstriction develops in both arterial and venous circulation The *function of this selective* vasoconstriction may be to secure a sufficient supply of oxygenated blood to working tissues such as the heart and other muscles

A haemodynamic pattern resembling chronic heart failure (i e unequal distribution of blood supply to various organs, increased utilization of oxygen in tissues, and an insufficient CO) may also be found in clinical circumstances with a diminished return of venous blood to the heart (*e g mitral* stenosis constrictive pericarditis), or when the amount of circulating blood and oxygen decreases, as well as in acute heart failure or peripheral circulatory failure (see Fejfar, 1958) A similar haemodynamic picture can be seen in severe

material) have been found (Edelman *et al*, 1950) Extracts of urine from patients with congestive failure contain anti diuretic materal (*Bercu, Rokaw and Massie, 1949, 1950*) with a great sodium retaining activity (Deming and Luetscher, 1950*a*,*b*), which disappears when the patients become compensated (Luetscher, Deming and Johnson, 1950, 1951) The substance responsible for this is aldosterone (Luetscher and Johnson, 1954) An increased excretion of aldosterone is not characteristic only of congestive failure, but accompanies nephrotic and cirrhotic oedema as well A permanent increase of aldosterone under these conditions is called secondary aldosteronism (Conn, 1955, Bartter, 1956, Milne and Muehrcke, 1956, Thorn *et al*, 1956, Liddle, Duncan and Bartter, 1956, Wolff, Koczorek and Buchborn, 1957)

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t al, 1953) The effect of

ing to Bartter (1956) the

th Addison's disease did

A high central venous pressure and a secondary excretion of humoral substances like aldosterone complicate the response

Werkö and co-workers (1955), in a study of systemic and renal haemodynamic changes in 146 subjects with different cardiac disorders came to a similar conclusion Their results suggest that "the adrenergic impulses could contribute to the diminished renal blood flow in severe heart disease before any signs of congestion are apparent" They think one of the factors causing the release of adrenergic impulses may be a decreased stroke volume

The origin of the afferent impulses of this functional haemodynamic reflex is not known There are, of course, several pieces of evidence on the influence of nervous impulses on diuresis Viar and co workers (1951) demonstrated an increase in urine flow and excretion of sodium as the result of a rising venous pressure in the head (following the compres sion of neck by a manometer cuff) Cort (1953), in agreement with these results found an increased diuresis with higher elimination of sodium in subjects with the head lowered (Trendelenburg position of 15°) The changes in renal blood flow were not reported Cathcart and Williams (1955) did not confirm this

Gauer and co workers (1954) described an increase in urine flow in anaesthetized dogs during the negative pressure breathing period This was also found in healthy human subjects (Sieker Gauer and Henry, *1952, 1954*) The rise in diuresis was not accompanied by increased elimination of electrolytes (Na^+ or K^+) This water diuresis was thought to be caused by stimulation of volume or stretch receptors localized in the cardiovascular system in the thorax (left atrium or pulmonary veins) The values of renal plasma flow were not measured in these experiments. We do not know, therefore if the changes reported were produced by a direct influence on the renal tubules without any change ... haemod...

hy...

...re produced

muscular effort in healthy subjects It differs from that found in heart failure by an increase in CO and by vaso dilatation in the skin due to increased temperature

Haemodynamic changes in heart failure therefore do not represent a new and special adaptation of the organism to the

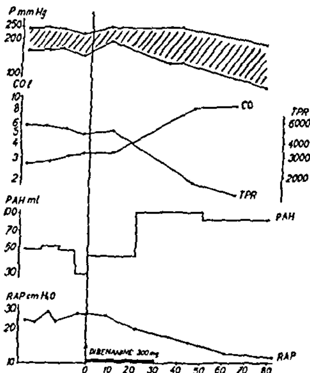


Fig 5 Changes in cardiac output (CO) peripheral vascular resistance (TPR) blood pressure (P) right auricular pressure (RAP) and renal plasma flow (PAH) after Dibenamine in a subject with heart failure See text for details (Fejfar Z (1957) *Acta cardiol (Brux)* 12, 18)

diminishing performance of the heart They are a typical reaction which appears in every situation in which CO is inadequate for oxygen requirement in the tissues This reaction becomes a chronic feature during the development of congestive failure and leads to retention of water and sodium

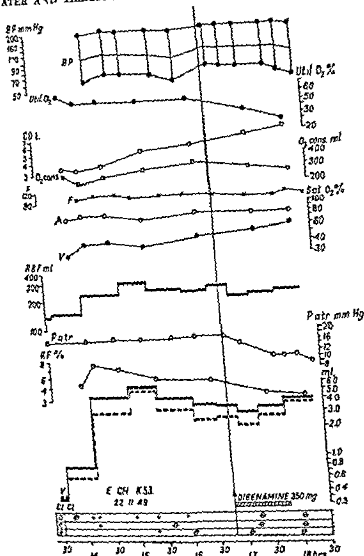


Fig. 6 Haemodynamic changes and renal excretion of chloride in a patient with acute pulmonary oedema. BP—blood pressure, F—pulse frequency Util. O_2—oxygen utilization in tissues (in percentage of O_2 supply) O_2 cons.—oxygen consumption/min CO—cardiac output, Sat. O_2—arterial (A) and mixed venous (V) oxygen saturation as a percentage RBF—renal blood flow RF—renal fraction of cardiac output; V—urine flow in ml/min Cl_{Cl}—chloride clearance, P atr—right auricular pressure, D—dyspnoea C—cough, R—râles.

evidence (see above) that renal blood flow and a decreased excretion of electrolytes occurs in left ventricular failure and mitral stenosis without right sided decompensation, when there is an increased pressure in the venous side of the pulmonary circulation

On occasion, however, a sudden increase of pressure in this part of the pulmonary circulation may be associated with a rise of urine flow in patients with a heart disease We have followed haemodynamic changes in nine patients with acute pulmonary oedema (Fejfar *et al*, *1958a*), in three of them we also studied renal haemodynamics and the excretion of electrolytes At the onset of recovery from pulmonary oedema there was a depressed renal blood flow and the renal fraction of CO started to increase before any significant changes in cardiac output occurred In two of these three patients the rise in renal blood flow was accompanied by an increased excretion of chloride (Fig 6) A rise of pressure in the left auricle and pulmonary veins is typical for acute pulmonary oedema in patients with mitral stenosis or left ventricular failure It is therefore possible that this elevation of pressure could influence renal blood flow, diuresis, and the excretion of electrolytes The diuresis was not, however, a water diuresis as described by Sicker, Gauer and Henry (1952, 1954)

Gömori and co workers (1954) studied renal circulation in dogs with crossed circulation under hypoxaemia They found a decrease in renal blood flow in a dog whose head was perfused from the other body by hypoxic (venous) blood Following denervation of the kidneys, this vasoconstriction either disappeared completely or was insignificant

Foldi and co workers (1955) found in hypoxaemic dogs a decrease in renal blood flow, excretion of water and electrolytes *In healthy subjects breathing a mixture of 10 per cent* oxygen there was also a decreased renal blood flow and elimination of electrolytes On the other hand a low renal blood flow, glomerular filtration rate and excretion of sodium significantly increased in patients with congestive heart

concentration of oxygen) CO rose in a similar way to the rise observed in hypoxaemic hypoxia in intact animals

(d) Harrison and co workers (1927) concluded from their studies on experimental hypoxaemia in dogs that the oxygen tension in the myocardium is the most important factor determining the rise in CO

A direct efferent nervous influence on the kidneys was demonstrated by Kaplan and Rapoport (1951) and Blake (1952) in dogs with unilateral renal denervation Tubular reabsorption of sodium was less in the denervated kidney Bykov and Alexejev Berkmann (1930, 1931) (see Bykov, 1952) found that a conditioned 'water' diuresis in dogs may be partly inhibited by denervation of the kidneys

Renal blood flow was measured only in the experiments of Kaplan and Rapoport (1951), where the increased renal excretion of water and electrolytes after splanchnicotomy was independent of changes in renal blood flow Our experimental results in patients with heart failure (see above) demonstrated a close relationship between changes in renal blood flow and tubular reabsorption of water and electrolytes

A partial answer to this question can be found in the experiments of Cort and Kleinzeller (1956) on isolated kidney tissues of rabbits Changes in transport of cations and water were studied during two hours exposure of kidney slices to unoxygenated [illegible]

[illegible] flux of sodium into the denervated slices during leaching at 0°, and a slower expulsion of sodium from the denervated kidney slices during the incubation period The changes in water content of the slices were in the same direction as the shifts of sodium The difference bet- [illegible]

[illegible] accumulation during subsequent incubation slower in the denervated kidney

failure inhaling 50 per cent oxygen plus 4 per cent carbon dioxide for 30 minutes (Foldi *et al*, 1956) According to these authors renal changes are brought about by hypoxia in the brain

It is improbable however that every case of heart failure is accompanied by cerebral hypoxia The renal changes are manifested, as shown above in left sided failure The results of Scheinberg (1950) indicate a decreased blood flow through the brain in heart failure together with a rise in cerebral vascular resistance If the cerebral supply of oxygen is really insufficient we might expect quite the reverse a diminution of cerebral vascular resistance and an increase in cerebral blood flow This was actually demonstrated in man during experimental hypoxaemia by Kety and Schmidt (1948)

We are of the opinion that the heart itself may be the starting point for the haemodynamic functional changes in heart failure and in all situations in which CO is inadequate for the requirement in tissues i e where oxygen utilization in tissues increases (Fejfar 1956 1957 1958) The basis for this hypothesis will be briefly summarized

(a) Myocardial utilization of oxygen is even with physical inactivity in healthy subjects greater than that by the other important organs of the body Every rise in oxygen consumption or utilization in tissues (muscular effort anaemia mitral stenosis etc) is associated with coronary vasodilation, an increase in the coronary fraction of CO and vasoconstriction in the kidneys

(b) We have demonstrated that during the inhalation of oxygen a normal CO in a healthy subject or in compensated patients either does not change or decreases while a low cardiac output in heart failure increases (*Fejfar* 1957 *Fejfar et al* 1958*a*)

(c) Gomori and co workers (1954) in experiments cited above did not find an elevation of CO during isolated hypoxia of the brain On the other hand when the isolated head of a dog was perfused by arterial blood and the trunk supplied with hypoxaemic blood (the dogs inhaled a mixture with a low

balance studies, and by analyses of muscle biopsies, that in addition to the cellular loss of potassium there is an increment of sodium in cells (Iseri, Boyle and Myers, 1950, Iseri *et al*, 1952, Squires, Crosley and Elkinton, 1951*a*, Warner *et al*, 1952, Cort and Matthews, 1954, see also Elkinton and Danowski, 1955, Cort and Fencl, 1957) Particularly important is the fact that potassium depletion occurs in subjects treated by repeated injections of mercurial diuretics (Squires *et al*, 1951*b*, Cort and Matthews, 1954) In some of these severely ill cases hyponatraemia and hypochloraemia with an elevated concentration of bicarbonate may be observed

Clinical diagnosis of potassium depletion in chronic congestive failure is difficult to prove Decompensated cardiacs excrete negligible amounts of sodium and the stronger acid radicals are excreted neutralized by potassium Therefore the typical finding of a far higher concentration of potassium than sodium in the urine in congestive failure is not alone sufficient proof of cellular loss of potassium

Plasma levels of Na^+, K^+, and Cl^- are usually within the normal range in decompensated cardiac patients

Table I presents the relationship between plasma levels of Na^+, K^+ and HCO_3^- and concentration of Na^+ and K^+ in muscle biopsy specimens in 13 patients with various degrees of heart failure Concentrations of total muscle Na^+ and K^+ are expressed in m equiv 100 g of fat free dry solids (FFDS) Normal values given by Cort (1955*b*) are about 13 ± 2 m equiv. of Na^+ and 45 ± 3 m equiv of K^+

It will be seen that all the patients had a decreased amount of potassium in skeletal muscle This K^+ depletion was very marked although not all were treated with mercurial diuretics Patient M E was not yet in right sided failure In all patients with the exception of A Z, the plasma concentrations of Na^+ and K^+ were within the normal range In the majority the concentration of bicarbonate was slightly elevated None of them showed ECG changes typical of

In six rabbits with bilateral denervation the resting clearances of inulin and PAH were practically the same as in the rabbits without renal denervation (Brod and Sirota, 1949) Cort and Kleinzeller (1956) therefore conclude that the differences described are due to a direct nervous effect on tubular cells rather than to a change in renal blood flow.

It is difficult to compare results obtained from experiments with tissue slices or in anaesthetized animals, with results from human subjects, in which every disturbance of homeostasis is immediately compensated for in several ways Neural and humoral regulation act simultaneously and it is practically impossible to differentiate them It seems, nevertheless, that even in subjects with chronic heart failure, retention of electrolytes and water is the result of haemodynamic changes parallel with increased tubular reabsorption of sodium and water These changes may be initiated by a reflex mechanism acting through adrenergic nerves Increased secretion of aldosterone and ADH is a secondary manifestation This secondary aldosteronism may, however, prevail in the long run, dominate the whole picture of chronic congestive failure, and close the vicious circle

Further consequences of retention of salt and water in heart failure

The retained sodium and water in congestive failure does not enlarge the volume of extracellular fluid only In patients recovering from heart failure the reduction of body weight was greater than the reduction in the amount of extracellular fluid (Seymour *et al*, 1942), chloride output (Schroeder, 1950) or sodium loss (Miller, 1950, 1951) This surplus water must come from cells In the development of congestive failure, the water accumulates in both extracellular and intracellular compartments

At the same time changes begin in the concentration of [illegible] electrolytes The loss of cellular [illegible] was described in 1930 by [illegible] It has been ascertained by

balance studies and by analyses of muscle biopsies, that in addition to the cellular loss of potassium there is an increment of sodium in cells (Iseri, Boyle and Myers, 1950, Iseri *et al*, 1952, Squires, Crosley and Elkinton, 1951*a*, Warner *et al* 1952, Cort and Matthews 1954, see also Elkinton and Danowski, 1955, Cort and Fencl, 1957) Particularly important is the fact that potassium depletion occurs in subjects treated by repeated injections of mercurial diuretics (Squires *et al*, 1951*b*, Cort and Matthews, 1954) In some of these severely ill cases hyponatraemia and hypochloraemia with an elevated concentration of bicarbonate may be observed

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Plasma levels of Na^+, K^+ and Cl are usually within the normal range in decompensated cardiac patients

Table I presents the relationship between plasma levels of Na^+, K^+ and HCO_3 and concentration of Na^+ and K^+ in muscle biopsy specimens in 13 patients with various degrees of heart failure Concentrations of total muscle Na^+ and K^+ are expressed in m equiv 100 g of fat free dry solids (FFDS) Normal values given by Cort (1955*b*) are about 13 $\pm$ 2 m equiv of Na^+ and 45 $\pm$ 3 m equiv of K^+

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Table I

RELATIONSHIP BETWEEN PLASMA LEVELS OF Na^+, K^+ AND HCO_3^- AND CONCENTRATION OF Na^+ and K^+ IN THE SKELETAL MUSCLE

MS—mitral stenosis; MI—mitral incompetence, Tri S—tricuspid stenosis, Tri ins—tricuspid insufficiency; H + I H D—hypertensive and ischaemic heart disease. See details in text.

Name	Sex	Diagnosis	Age years	Degree of heart failure	Muscle: Na^+ total m-equiv./100 g FFDS	Muscle: K^+ total m-equiv./100 g FFDS	Plasma: Na^+ m-equiv./l.	Plasma: K^+ m-equiv./l.	Plasma: HCO_3^- m-equiv./l.	Note
F Č	M	MS>MI Tri S	33	5	10.23	18.8	137	4.47	23.3	
A S	F	Atr. sept. d. f.	48	3–4	19.75	27.6	141	5.15	29.4	0 mercurial diuretic
M B	F	MS	38	4	13.9	28.3	150	4.54	28.0	
M P	F	MI>MS	37	3	15.04	28.64	137	4.5	26.4	0 mercurial diuretic
I D	F	MS>MI postcommis	40	4	23.39	21.52	145	5.75	23.1	0 mercurial diuretic
M D	F	MI, bacterial endocarditis	35	3	12.51	29.92	143	3.9	30.2	0 mercurial diuretic
E K	M	MS Tri S	46	5	14.3	37.2	131	5.34	28.5	
P U	M	MS Tri ins.	43	5	27.1	25.46	144.1	4.56	23.1	
A Z	M	MS postcommis	49	5	22.5	11.2	126.5	4.02	14.8	8th day post operative
A N	F	MS	51	3	10.3	21.76	149.5	5.2	31.1	
H Ch	F	MS postcommis	37	4	19.57	33.61	143.3	4.07	23.5	
N B	M	H + I H D	60	4	16.91	39.89	143.5	4.09	29.4*	* not at the same time
M N	F	MS postcommis.	37	3	20.78	34.06	141.5	4.44	26.8	

was found in patient A Z, with suppuration in the thoracic wound one week after mitral commissurotomy, 24 hours before death. He was by this time in severe metabolic acidosis. The loss of about three quarters of the muscle potassium was

the probability of being hit according to the number of times the object has been hit before, and if you proceed further and increase the speed of the shooting the mathematics develop in the direction of the Makeham curve and certain modifications of it

Maynard Smith I do not believe for one moment that the shape of these curves has anything to do with the organism that has been studied I think it has something to do with the environment in which it was studied

Perks I have thought about this problem for a long time and I believe the shape has something to do with time

Tanner You would expect a different-shaped curve in a less long lived organism?

Perks No I think it is the cumulative factor in life injuries and so on are additive or cumulative in almost a geometrical sense and you must expect to have exponentials coming into the form of the mortality rates

Tanner Does this make the change then from the gamma function into the Makeham type of curve? You think that the reception state changes according to the number of shots impinging?

Perks Yes

Holt In the models for fitting the depreciation of motor cars death is assumed to result from either single big accidents or an accumulation of small ones and a complex death curve follows it seems to have some possible application to animal mortality

Rotblat I am concerned to know what lifespan is because we have to express some of our findings in terms of lifespan For example people often speak about the effect of radiation in causing a shortening of the span of life and they put down figures of the percentage of life shortening per rontgen But everyone I have asked what he meant by the term lifespan gave a different answer I was hoping that perhaps in this meeting we might come to some agreement on that I was impressed by your remarks on the first day Dr Benjamin when you brought in what you called the senescence peak This peak seems to me to be the quantity to put down as the normal lifespan I was particularly interested to see data presented by various people which showed that although the shapes of the mortality curves may differ enormously nevertheless for a given species they all reach nearly the same end point If we could draw the ideal curve i e if we could eliminate all deaths due to accidents infections etc then the normal lifespan would be obtained immediately from the senescence peak But in practice this will be very difficult because most mortality curves do not approximate to this ideal We are therefore still left with the question of what we

ertain extent by the infantile mortality, and by mortality from ents and infections. If you reduced those mortalities, then, as a piece of arithmetic, the mode is advanced and the curve of

pendent.

Rockstein: I think we are being unrealistic about this. I would ggest that mean longevity seems to be a thing that you could

obtain ...
cerned ...
pare, why *not* use an animal, as ... which you can get such data? I have been amazed that ... generations in the housefly, for example, *one can* continue to *get the* same *mean value. I say* this value is a *good* basis for *comparison in* an experimental study. With the white rat, under specific laboratory conditions, for example, the *only thing* that will vary in irradiation ... or dosage of *radiation.* With humans we run ... with life expectation ...

inbred *lines* of a numbe... why the mean *longevity* is not as good a criterion ... it considers the accidental deaths, it considers the possible disturb... would have from having had early deaths or ... resulting from the extended long...

Perks: I am sure you are right ... it different *problems* require different solutions.

Rotblat: If we say, for example, that radiation causes *a contraction* of the *scale* of life, and suppose we are dealing with the ideal case in which all animals die of old age, then for the irradiated animals we *would* obtain ... curve but bodily shifted to the left. This would be very ... It may be that radiat... The ... actually ... lifespan.

... average is ... *the first quantity* ... ex... the effects of ... terms of a st... ing ... of the survival curve are ... mental factors under investigation. I have ... studies of radiation effects *on mice*, you could characterize the effects ... of the *A and* the *α parameter of the* Gompertz ... radiation—to restate what I ... without a change in *α*. Con... *It is* perfectly true that this Gompertz ... adequate description of the life-*tables* of natural popula... it should be

borne in mind th
cussing general pr
expressions are us

is

$$q_x = \sum_{i=1}^{n} A_i e^{b(x)}$$

Perks. If you are fitting mathematical expressions to your data of statistical distribution, then clearly you estimate the parameters, and your estimated values for the parameters sum up the statistics. I thought the problem we were really talking *about* was how to characterize statistics for which you have not got a mathematical e . . For a single measure to be sufficient the distribution

can say is that for some purposes one measure may be better than another. You have to accept that the expectation of life or the half-

curve that you can really say that any parameters are better estimators than any others.

Benjamin. All this discussion of lifespan seems to be only a means to an end, we really want to get away from lifespans to considering the ageing effects, for example, of changes in environment. For that we really want two things. First, we need some kind of function

may make a difference between the curve bulging up one way, or bulging down the other. The largest possible dispersion of effects is found here, which brings us to the idea of the 50 per cent survival age point. To get a time scale which enables comparisons to be made between different kind [illegible] on the other hand, you need something which is not [illegible] This suggests that you should use [illegible] although the height of the peak is very much corr[illegible] pated deaths and so on, the actual movement of the peak is not particularly sensitive So while it is unprofitable to talk about an ideal lifespan, it is quite practical and profitable to look at large families of curves of death for the same species, and see what kind of shape they tend to in general, so that you can get for the different species a typical modal attained length of life, which you could use as the time scale

Comfort This is Bodenheimer's "physiological longevity" (Bodenheimer, F. S. (1938). Problems of animal ecology Oxford [illegible] Press)

[illegible] you should all take an interest in [illegible] I think it is a very good thing that all scientists should [illegible] notation if there is one which is generally accepted Dr Benjamin has included some of it in his paper and I think it probably could be extended to cover all your needs

We have heard the phrases lifespan and ageing over and over again but I do not think you will ever succeed in rigorously defining them I think they are best left as rather vague concepts, as we know in general what we are talking about

I have been a little puzzled by the extent to which logs have been taken of various observed figures To me it only confuses the issues, particularly when you have a graph on a logarithmic scale, although I do understand that sometimes it is necessary to do that to compress the graph to reasonable dimensions But there is no excuse for taking logs in arithmetic merely to get rid of some of the variations, and apparently to produce a correlation or regression which possibly is not there if you do not take logs What does the logarithm of a residual mean? When you take a logarithm of the cephalization index and then associate it with a logarithm of body weight, and [illegible] with a logarithm of a lifespan, what does it all mean?

[illegible] technique that might be useful for [illegible] of animals in their [illegible]

but [illegible]

state So if you take the crude death rate over an interval of time, then a fair measure of the expectation of life, in terms of that interval, is the reciprocal of the crude death rate, provided that population is stationary, or nearly stationary. If it is not nearly stationary, you can probably make an approximate adjustment

The only other thing I want to say is that the actuary's use of life-tables is very different from yours The life table is not an end in itself for actuaries, it is merely a step on the way from a set of mor[illegible] the calculation of premium rates, reserves, bor[illegible]

[illegible] They are ac[illegible] al[illegible] as [$Y = ax^b$] [illegible] these allometric relations are almost [illegible] on a double logarithmic scale When you take the log [illegible] lo[illegible] then a linear relationship between these values T[illegible] logarithms to fit a power function [illegible] to brain

[illegible] is p[illegible] ne[illegible]

[illegible] th[illegible] ap[illegible] tr[illegible] an[illegible] therefore the error in logar[illegible] though the original absolute values have a minimum [illegible] f variation—from 5 grams or so to 5 000 kilograms.

Perks I agree that if you have reason for a relationship of that f[illegible] may ease the arithmetical processes [illegible] of a general theory of [illegible] capable [illegible] this form It is however, a fact [illegible] functi[illegible] the these relations, and no other functio[illegible] t as we[illegible]

Co[illegible] and [illegible] mortal[illegible]

may make a difference between the curve bulging up one way, or bulging down the other. The largest possible dispersion of effects is found here, which brings us to the idea of the 50 per cent survival age point. To get a time scale which enables comparisons to be made betwe[illegible] of animals, on the other hand, you need someth[illegible] of change. This suggests tha[illegible]
although the height of the [illegible]
pated deaths and so on, the actual movement [illegible]
particularly sensitive. So while it is unprofitable to talk about an ideal lifespan, it is quite practical and profitable to look at large families of curves of death for the same species, and see what kind of shape they tend to in general, so that you can get for the different species a typical modal attained length of life, which you could use as the time scale.

Comfort: This is Bodenheimer's "physiological longevity" (Bodenheimer, F. S. (1938). *Problems of animal ecology*. Oxford [illegible]

[illegible] ou should all take an interest in the [illegible] I think it is a very good thing that all scientists shou[illegible] ie notation if there is one which is generally accepted. Dr Benjamin has included some of it in his paper and I think it probably could be extended to cover all your needs.

We have heard the phrases lifespan and ageing over and over again but I do not think you will ever succeed in rigorously defining them. I think they are best left as rather vague concepts, as we know in general what we are talking about.

I have been a little puzzled by the extent to which logs have been taken of various observed figures. To me it only confuses the issues, particularly when you have a graph on a logarithmic scale, although I do underst[illegible] it is necessary to do that to compress the grap[illegible] is no excuse for taking logs [illegible]
and apparently [illegible]
is not there if you do not take logs [illegible]
residual mean? When you take a logarithm of the [illegible]
index and then associate it with a logarithm of body weight, and finish [illegible] a logarithm of a lifespan, what does it all mean?

Th[illegible] technique that might be useful for those [illegible] of animals in their natu[illegible]
but ofte[illegible]

That seems to
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in man than is ...
what Dr Chitty has said I feel like withdrawing this comment, because if in other species the probability of their dying in the field increases with age, this is the fundamental thing It comes back to

use an arith /log scale, arith for time and log for survival, so that constant mortality gives a straight line

Tanner I have been sitting here for the last half hour with a very strong feeling of *déja vu* The people who are interested in growth have been fitting growth curves with decreasing enthusiasm for about 35 or 40 years It seems to me that you are pursuing a vertiginous and descending pathway!

I do not think there is anything in the general aspects of growth which leads one to suppose that the allometric relationship is very useful in general There may be instances where it is necessary, not from any theoretical considerations but because using logs produces a straight line, I do not think that there can be any other justification

Sacher A transformation such as the logarithmic cannot increase the amount of information contained in a set of data so if the correlation of the transformed variables is 0 99+ this expresses a fact about the data i e that only a fraction of a percentage of the total variance is error variance when the proper functional relation between the variables is found No *a priori* justification is needed for the use of the power function In my own and Brody s data there is no question about its appropriateness The fact that some other data are adequately rectified by a linear plot is interesting but it cannot contravene the allometric relations as they have been established in many other cases

Chitty My particular problem is to find out why animal populations in nature do not go on increasing indefinitely and what it is that they die of Most people up to the present have considered that deaths in nature could be almost entirely accounted for through predation or epidemic disease heavy infestation with parasites or food shortage but it is now clear that this is a wholly inadequate explanation particularly for the huge mortalities which occur in the young stages The problem arises what exactly is it that they die of? The suggestion was first made by P H Leslie and R M Ranson in 1940 (*J Anim Ecol* 9, 27) for the field mouse that the life table type of explanation might be applied to field populations In the laboratory you recognize that with age there is an increasing probability of death from a variety of causes which are peculiar to the particular environments—that is to say a group of mice in one laboratory would not have the same final causes of death as they would in another—but in each case there would be the common fact that as they grew older they became increasingly liable to die of whatever it was that was peculiar to those environments The field evidence strongly suggests that this may be a profitable way of

looking at natural populations, too The problem now becomes to try and find some general law which is applicable in spite of the fantastic variety of conditions in the field. I think one would never hope to find any common causes of death associated with the actual time of death in nature Every environment differs in its hazards from every other one The point is can we find anything in the properties of the animals which does obey some sort of general law under all these varied circumstances? In other words what is it that makes an animal increasingly likely to die regardless of what actually kills it in the end? We might divide the problem into both multiple and single processes The final causes of death would be the multiple processes and there we have very little hope of introducing much unity but by concentrating on susceptibility there may be some hope of finding a common process which can equally well be studied in those animals which live to a great age in the laboratory and those which die at a much younger age in nature Exactly how one goes about this I do not know and that is the point at which my work is hung up I have to account for very violent changes in the probability of survival at different times of the population cycle, and exactly where does one go to look in the organism for something which may be an index of this change in properties? That seems to me to be very similar to the problems with which one is faced in t[illegible]count for the increasing probability of death with age [illegible] to an age at which you can say t[illegible] even at a much [illegible]

and Ev[illegible] question [illegible] of senes[illegible] probabi[illegible] and some index of physiologic[illegible] trying to find in any species

Tanner There does not seem to me to be any connexion between the situation in non-domesticated animals and the situation in the human All the other mammals and birds would have died early in terms of human growth The human was the only animal which seemed to be surviving long enough to experience senescent processes Cellular ageing might paradoxically have been closer to the situation in man than is the situation in the passerine for example After what Dr Chitty has said I feel like withdrawing this comment because if in other species the probability of their dying in the field increases with age this is the fundamental thing It comes back to

use an ... log scale, arith for time and log for survival, so that consta ... straight line

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senescence The nature of this grouping probably has some moral significance

A point that impressed me very much was the difficulty of evaluating the significance of data obtained from animals in captivity which are living in conditions of constant diet, no exercise constant illumination very little in the way of seasonal change, and so on, to which even animals which have been selected for laboratory purposes are not really 100 per cent adapted After all the period of adaptation to laboratory life is comparatively short compared with the period of evolution I do not really know what can be done about that What is outstandingly important is that wherever possible a pathologist should look at the animals when dead and this should be done for insects as much as for any other form of animal life.

The concept of a biological time scale continues to interest us though it is obviously even vaguer than some of the other concepts which, as Mr Perks remarked are better kept vague However, this particular one is of no use to us unless we can measure it It is useless to continue to use it without exact definition

Whatever the source if we exclude accidents to which the individual concerned does not contribute in most instances susceptibility to mortality in a species or a strain does develop in a typical manner It is perf ... has been said that if one cause of mortality ... ch we have had data ana ... would r ... have survived ... eliminating one cause of death is not ... tionally we *appear* to be dealing with a unitary process ... ageing is in fact unitary however cannot be determined from the d ... n presented with so far I am not at all clear to w ... of synchronization of inde p ... a very

... the energy ... one but ... value it ... at a rate ... this is q ... open to ... study o ...

We ought to use metabolic poiso ...

what I was trying to say before, that just as in growth we talk about developmental age so we can talk about the probability of death in any given situation as developmental age further down the scale If that is really true, if these analogies can be made the same in animals and man then we can use the results of both field workers and the experimentalists much more usefully to shed light on the human situation

Comfort The figures we get for small mammals have often misled people into thinking that all mammals behave in this way We have only very few figures for the larger ones but there is some evidence that many larger mammals in the wild achieve high ages quite often I would be very surprised if some of the larger wild ungulates didn t live long enough to senesce Wild horses if one had the chance of observing them may perhaps live to a fair proportion of the age reached by tame horses

Sacher Porcupines can survive until they are so arthritic they can hardly climb trees—nobody comes up against them except mountain lions

Verzár Dr Chitty did you imply that the time of survival for a certain species depends on the number of individuals living at the time? If you put a pair of fish in a pond they will have certain survival characteristics for their age When the pond becomes full with fish the population will become constant Then the individual survival curve will probably be different otherwise the number of fish would continue to increase which is impossible because there is not enough food

Chitty The number alive has a very great influence on the survival rate but the survival time can certainly not be predicted from the numbers of the animals alone One must also take into account the behaviour of the population experimentally you can have a family group of 50 animals which is perfectly amicable and has a good survival rate yet with two animals in a similar space who are strangers the survival time of one of them will be about half a day The presence of other hostile animals of the same sort is I think one of the strongest environmental factors which does affect survival regardless of the amount of food available

* * * *

Danielli I shall not attempt in any way to summarize this meeting I have been considerably refreshed by it in many ways We have learned for example that man skunks and porcupines are amongst the few animals who know how to survive into the period of

senescence The nature of this grouping probably has some moral significance

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have survived The total gai[illegible] is not great In ot[illegible] eliminating one cause of death is not great In ot[illegible] tionally we *appear to* be dealing with a unitary process Whether ageing is in fact unitary however cannot be determined from the data we have been presented with so far I am not at all clear to [illegible] Smith's theory of synchronization of inde[illegible] I am sure a very

[illegible]

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study of hu[illegible]
We ought [illegible]

desynchronize some of the processes which may be synchronized. It may be in this connexion that we could make some progress by studying systems in which one is normally dealing with symbiosis. *Here the breaking-up of the symbiotic relationship might reveal* phenomena which would be difficult to reveal by any other method.

A cellular approach to ageing would under certain circumstances facilitate examination of the actual process of ageing, if this is in fact to some degree unitary, but I am not convinced that this is so with higher animals. The ageing process in higher animals may be fundamentally a function of the complex of cells and operate at a higher level of organization than is present in cells.

Another point which we have not discussed yet, but which we are probably all agreed upon, is that it is quite possible that lifespan in man, as we see it now, is an entirely accidental by-product of selection for breeding efficiently at a much earlier age, and does not in any sense correspond to social needs of the moment. An accumulation of experience has become much more important, or at any rate equally as important as physical vigour, therefore socially speaking there is a very good case for anything which will enable us to modify the expectation of life in a radical manner. Looking at this from the long term point of view, I think the possibilities for so doing would in fact be good by chemical means, provided ageing does in fact occur by a unitary process. If on the other hand it is due to a very large number of non unitary processes which do not have any common mechanism, then I think the chemical approach to extension of lifespan is fraught with so many difficulties that it is hardly worth considering. To illustrate the order of magnitude which I would expect if there is a unitary process involved, I would like to refer briefly to the rate of mutation, or rather the rate at which damage is caused by radiation. It is of course a tenable hypothesis that somatic mutation is one way or another directly concerned with the ageing process. There are cells which are killed by a dosage of the order of 100 r, there are other cells which require a dosage of 1 000 000 r to kill them. This difference of four orders of magnitude is not at present, as far as I know, accountable for in any cytological or physiological terms whatever. Therefore, if ageing were associ*ated primarily with somatic mutation, or some other generalized* mechanism of this type, we might expect that under suitable conditions lifespans could be varied by that order of magnitude. This sounds a little like science fiction, but all of us have seen so much of what we regarded as science fiction 20 years ago turning up as reality, that I think we must envisage the possibility that we may be able to make radical changes in lifespan.

My final comment is that I regard it as singularly unfortunate that so much of the research on cancer is conducted essentially without reference to the phenomena of ageing, of which I think it is a part. I believe that not only would a study of ageing benefit from more knowledge of what is going on in older animals, but the whole field of investigatic [illegible] ght benefit equally, and perhaps far more This fo [illegible] considerable degree, at the momer [illegible] plantable tur [illegible] then seeing what could be [illegible] does not approxi[illegible] conditions—a thing which does not approxi[illegible] what happens in the average human patient. Prof Muhlbock's laboratory is a shining example of an institute where this isolation of cancer research from ageing research does not occur, but I hope it will not long continue to be one of the very few shining examples.

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APPENDIX

NOTE ON SOME MATHEMATICAL MORTALITY MODELS

R E Beard

Pearl Assurance Co Ltd London

1 A satisfying basis for a law of mortality would be a formula that, starting from some fundamental concepts about the biological ageing process, led to a distribution of deaths by age which was comparable with observational data Such comparison would not be simple and straightforward because environmental and secular factors would introduce distortions as compared with the theoretical underlying distribution

2 In the course of numerical work extending over a number of years, on the expression of human mortality functions by mathematical formulae various attempts have been made by the writer to develop an approach on this basis The results obtained have not led to any satisfying formulae but they are suggestive of different lines of approach and have been summarized below in the hope they may be of value to others interested in the subject The note follows the sequence in which the ideas have developed in the mind of the writer and leads from considerations based on the force of mortality, μ_x, to those based on the curve of deaths $\mu_x l_x$

3 The first mathematical expression which provided a reasonable representation of the observed force of mortality in human data was that first proposed by Gompertz (1825) and later modified by Makeham (1867) Basically the law was derived by postulating a relationship between the rate of change of the force of mortality at any age and its value at that age The next significant modification to the Makeham law was the system of curves devised by Perks in 1932 and of which the important formula was the logistic Many human life-tables have been graduated by this basic curve modified in some instances to allow for special features of the data particularly at the younger and early middle ages and the clear fact emerges that adult human mortality can be very well represented by a logistic curve of the form

$$\mu_x - A = B\,c^x/(1 + D\,c^x) \qquad (1)$$

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1939a, 1951a,
Lives, 1956)
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quency curves which can be used to represent the distribution o

mortality tables as being appr
0 57 (Beard, 1951b)

are those arising from accide

time

Gompertz' law arises by using condition (c) only,

$$\text{i e}\quad d\mu_x/dx = \lambda\mu_x \qquad \text{whence}\quad \mu_x = B\,e^{\lambda x}$$

Makeham's law arises by using conditions (a) and (c),

$$\text{i e}\quad d\mu_x/dx = \lambda(\mu_x - A) \qquad \text{whence}\quad \mu_x = A + B\,e^{\lambda x}$$

Perks' law arises by using conditions (a), (b) and (c),

i e. $d\mu_x/dx = \lambda(\mu_x - A)(E - \mu_x)/(E - A)$ (4)

$$\text{whence} \quad \mu_x = A + \frac{(E-A)\,D\,c^{\lambda x}}{1 + D\,c^{\lambda x}}$$

The Perks (logistic) relation can be expressed as stating that the rate of change of μ_x is proportional to the product of its value and the amount by which it falls short of its upper limiting value

8 If the requirement of a constant upper limit for the rate of mortality is relaxed other formulae can be developed on similar lines to those of the preceding paragraph For example,

$$\frac{d\mu_x}{dx} = \frac{\lambda(\mu_x - A)}{1 + B(\mu_x - A)} \quad \text{gives} \quad w_x c^{w_x} = C\,c^{\lambda x} \qquad (5)$$

$$\text{where} \quad w_x = B(\mu_x - A)$$

and

$$\frac{d\mu_x}{dx} = \frac{\lambda(\mu_x - A)\left(1 + \frac{D}{B}\overline{\mu_x - A}\right)}{\left(1 + \frac{2D}{B}\overline{\mu_x - A}\right)} \qquad (6)$$

$$\text{gives} \quad \mu_x = A + \frac{B}{2D}\left(-1 + \sqrt{1 + 4\,D\,c^{\lambda x}}\right)$$

Formula (6) is equivalent to a continued fraction form for μ_x, i e

$$A + \cfrac{B\,c^{\lambda x}}{1 + \cfrac{D\,c^{\lambda x}}{1 +}}$$

and the relationship between formulae (2) to (6) is clearly seen by expanding the expressions for μ_x in terms of powers of $c^{\lambda x}$, i e

formula (2) gives $B\,c^{\lambda x}$

" (3) " $A + B\,c^{\lambda x}$

" (4) " $A + B\,c^{\lambda x} - BD\,c^{2\lambda x} + BD^2\,c^{3\lambda x}$ —

" (5) " $A + B\,c^{\lambda x} - BD\,c^{2\lambda x} + \frac{3}{2}\,BD^2\,c^{3\lambda x}$ —

" (6) " $A + B\,c^{\lambda x} - BD\,c^{2\lambda x} + 2\,BD^2\,c^{3\lambda x}$ —

9 The differences between formulae (4), (5) and (6) will become apparent only at the old or very old ages and unless the data were extensive the differences would be unlikely to be significant for many numerical processes From a scientific point of view the models are, of course, quite different

10 An alternative approach to the question, but still based upon rates of mortality, is to determine the conditions necessary for μ_x to be a Perks (logistic) curve, given that the population can be stratified according to a longevity factor and that the basic mortality law is Makeham in form (Beard, 1952b) Thus let μ_k^s be the force of mortality at time ($\equiv$ age) k for the group having longevity factor s and let $\phi(s)\,ds$ be the proportion of the initial population having factor s Then the survivors of $\phi(s)\,ds$ at time k are

$$\phi(s)\,ds\;\exp\left(-\int_0^k \mu_t^s\,dt\right) \tag{7}$$

and the total survivors at time k

$$l_k = \int \phi(s)\exp\left(-\int_0^k \mu_t^s\,dt\right)ds \tag{8}$$

where the integral is taken over the whole range of s
The force of mortality at time k $(= -d\log l_k/dk)$ is then

$$\mu_k = \frac{\int \phi(s)\,\mu_k^s \exp\left(-\int_0^k \mu_t^s\,dt\right)ds}{\int \phi(s)\exp\left(-\int_0^k \mu_t^s\,dt\right)ds} \tag{9}$$

11 From formula (9) it will be noted that μ_k is a weighted mean of μ_k^s $(= \mu_k^{\bar{s}}$ say) Since the number of lives with heavier mortality will diminish more rapidly than those with lighter mortality, $\bar{s}$ will decrease with increasing k If the basic mortality is Makeham in form then $d\mu_k/dk$ will show a slackening off at the higher ages, i e. the sigmoid feature shown by a logistic curve In order to meet practical conditions some limitations are necessary on the form of $\phi(s)$ the lower limit must be $\geqslant 0$, but the upper limit can be ∞.

12 If it be assumed that $\phi(s)$ is a gamma function such that $\phi(s)\,ds = ks^{\nu}e^{-\gamma s}\,ds$ $(0 \leqslant s < \infty)$ and that the mortality function for $\phi(s)$ is $\mu_k = \alpha + \beta s\,e^{\lambda k}$ we have

$$\mu_k = \frac{\int_0^\infty ks^{\nu}e^{-\gamma s}(\alpha+\beta s\,e^{\lambda k})\exp\left(-\int_0^k(\alpha+\beta s\,e^{\lambda t})\,dt\right)ds}{\int_0^\infty ks^{\nu}e^{-\gamma s}\exp\left(-\int_0^k(\alpha+\beta s\,e^{\lambda t})\,dt\right)ds} \tag{10}$$

which reduces to

$$\mu_x = \alpha + \frac{(p+1)\beta\lambda c^{\mu}}{(\gamma\lambda-\beta)+\beta c^{\mu}} \quad (11)$$

which is a Perks (logistic) form

13. The results of the immediately preceding paragraphs are interesting in that the limiting value of μ_x arises from the manner in which the '*mixed*" population runs off They have a certain appeal in that they are based on the assumption that the population is not homogeneous in regard to a mortality (or *longevity*) factor and that the mortality for an individual group continues to increase indefinitely The limiting value of $\mu_x-\alpha$ as $\lambda\to\infty$ from formula (11) is $(p+1)\lambda = 4\lambda/\beta_1$ where β_1 is the Pearson moment function of $\phi(s)$ For human lives $\mu_x \sim 0$ 6 at the limit according to one fairly recent mortality table and $\lambda \sim 0$ 1 so that $\beta_1 \sim 0$ 67 i e a skew distribution with a tail towards *the higher values of s If s is* a heredity factor, then stability of $\phi(s)$ over *generations* would imply fertility rates negatively *correlated* with longevity otherwise *the* shorter reproductive period of those with higher values of *s* would lead to a falling average value *of s* in the population It is an *interesting* coincidence that the distribution of *married* women according to number of children born has a β_1 coefficient of *the* order of 0 7 (Papers of Royal Commission on Population 1950)

14 The assumption of other forms for $\phi(s)$ in formula (9) leads to other forms for μ_x which can *have* the appropriate shape *but* which are not convenient mathematically and no experiments have been made in this direction

15 From the point of view put forward *in* paragraph 1 formula (10) suffers from *the* objection that it is based on the assumption of a Makeham law, and is thus basically empirical A further approach to the question is to build up models based on the so called 'shot hypothesis' in which individuals are assumed to be subject to random firings *and* are assumed to die when *they* have been hit a specified number of times Two main types of model have been investigated, which are referred to below as the 'forward' and "backward' models respectively In the forward model hits are assumed to accumulate and death to *occur* when the total reaches a certain figure *In* the backward model the individual *is* assumed to start with a quota of units which are progressively lost in time death occurring when the total remaining falls below a certain figure

16 The simplest forward model is derived by assuming that the

chance that an individual is hit in an interval dt is p; this leads to a difference-differential equation

$$\frac{dl_t^\alpha}{dt} = -pl_t^\alpha + pl_t^{\alpha-1} \tag{12}$$

where l_t^α represents the number at time t who have been "hit" α times. If l_o is the number of individuals at time o then a solution of equation (12) is

$$l_t^\alpha = l_o e^{-pt}(pt)^\alpha/\alpha! \tag{13}$$

If the number of hits causing death is r, then the survivors at time t are

$$l_t = l_o e^{-pt}\{1+(pt)/1!+ \ldots +(pt)^{r-1}/(r-1)!\}$$

and the deaths in the interval t to $t+dt$

$$\mu_t l_t = l_o e^{-pt} p^r t^{r-1}/(r-1)! \tag{14}$$

The force of mortality at time t is

$$\mu_t = \frac{p^r t^{r-1}}{(r-1)!} \bigg/ \left\{1+\frac{pt}{1!} \ldots + \frac{(pt)^{r-1}}{(r-1)!}\right\}$$

$$= pe^{-pt}(pt)^{r-1} \bigg/ \int_{pt}^{\infty} e^{-x} x^{r-1}\, dx \tag{15}$$

Formula (15) shows that the curve of deaths is an incomplete gamma function, or a Pearson type III curve. μ_t has the value 0 for $t = 0$ and asymptotes to a value p at $t = \infty$ (Beard, 1939b).

17 A more natural function than μ_x in the present context is to use the function which bears the same relationship to $\mu_x l_x$ as μ_x does to l_x, i.e.

$$\frac{d(\log \mu_t l_t)}{dt} = \frac{1}{\mu_t}\frac{d\mu_t}{dt} - \mu$$

and from formula (14) we find this to be

$$\frac{d(\log \mu_t l_t)}{dt} = -p + \frac{r-1}{t} \tag{16}$$

18 Attempts to use the formula of paragraph 16 on human mortality data have been unsuccessful, the shape of $d(\log \mu_t l_t)/dt$ not

which reduces to

$$\mu_x = \alpha + \frac{(p+1)\beta\lambda c^{\lambda x}}{(\gamma\lambda - \beta) + \beta c^{\lambda x}} \tag{11}$$

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13. The results of the immediately preceding paragraphs are interesting in that the limiting value of μ_x arises from the manner in which the "mixed" population runs off They have a certain appeal in that they are based on the assumption that the population is not homogeneous in regard to a mortality (or longevity) factor and that the mortality for an individual group continues to increase indefinitely *The* limiting value of $\mu_x - \alpha$ as $k \to \infty$ from formula (11) is $(p+1)\lambda = 4\lambda/\beta_1$ where β_1 is the Pearson moment function of $\phi(s)$ For human lives $\mu_x \sim 0\ 6$ at the limit according to one fairly recent mortality table and $\lambda \sim 0\ 1$ so that $\beta_1 \sim 0\ 67$ i e a skew distribution with a tail towards the higher values of s If s is a heredity factor, then stability of $\phi(s)$ over generations would imply fertility rates negatively correlated with longevity otherwise the shorter reproductive period of those with higher values of s would lead to a falling average value of s in the population It is an interesting coincidence that the distribution of married women according to number of children born has a β_1 coefficient of the order of 0 7 (Papers of Royal Commission on Population 1950)

14 The assumption of other forms for $\phi(s)$ in formula (9) leads to other forms for μ_x which can have the appropriate shape but which are not conveniently mathematically and no experiments have been made in this direction

15 From the point of view put forward in paragraph 1 formula (10) suffers from the objection that it is based on the assumption of a Makeham law and is thus basically empirical A further approach to the question is to build up models based on the so called "shot" hypothesis in which individuals are assumed to be subject to random firings and are assumed to die when they have been "hit" a specified number of times Two main types of model have been investigated which are referred to below as the "forward" and "backward" models respectively In the forward model hits are assumed to accumulate and death to occur when the total reaches a certain figure In the backward model the individual is assumed to start with a quota of units which are progressively lost in time death occurring when the total remaining falls below a certain figure

16 The simplest forward model is derived by assuming that the

the solution of which leads to

$$l_t^\alpha = l_0 e^{-p(\omega-t)} \{p(\omega-t)\}^\alpha/\alpha! \tag{23}$$

from which

$$\frac{d(\log \mu_t l_t)}{dt} = p - \frac{\alpha-1}{\omega-t}$$

if the deaths occur at the αth hit In this formula $p \sim 0\ 3$, $\alpha \sim 11$ and $\omega \sim 110$ for human mortality

22 No obvious physical model applies to equation (22), but the relationship can be written in the backward form

$$\frac{dl_t^\alpha}{dt} = -\frac{\alpha}{\omega-t} l_t^\alpha + \frac{\alpha+1}{\omega-t} l_t^{\alpha+1} \tag{24}$$

in which the rate at which a unit is lost is proportional to the number of units remaining divided by the years of life remaining to the final age ω From a biological point of view the concept of a final age by which the organism must be dead is unsatisfactory, but the fact that satisfactory numerical results arise only from a backward formula suggests that a closer study of this type of model might be more profitable

23 The simplest backward model arises from the relationship

$$\frac{dl_t^\alpha}{dt} = -pl_t^\alpha + pl_t^{\alpha+1} \tag{25}$$

where the organism is assumed to lose a unit at rate p.
This has a solution

$$l_t^\alpha = l_0 e^{-pt} (pt)^{n-\alpha}/(n-\alpha)! \tag{26}$$

where n is a maximum number of units If death is assumed to occur when the number of units falls below r, we have

$$\frac{d(\log \mu_t l_t)}{dt} = -p + \frac{n-r}{t} \tag{27}$$

This is of similar form to equation (16) and is not suitable for human data.

24 By assuming that the rate of loss of a unit is proportional to the number of units remaining the relation

$$\frac{dl_t^\alpha}{dt} = -p(\beta+\alpha) l_t^\alpha + p(\beta+\alpha+1) l_t^{\alpha+1} \tag{28}$$

fitting well to observed values which show a negative second differential coefficient over the adult ages.

19. As an extension of formula (12) a model can be set up in which the "hits" in an interval can be single, double, etc, in known proportions. The basic relation then takes the form

$$\frac{dl_t^x}{dt} = -pl_t^x + p\sum_{r=1} f(r)\, l_t^{x-r} \qquad (17)$$

This can be integrated to

$$l_t^x = e^{-pt} \int pe^{pt} \sum f(r)\, l_t^{x-r}\, dt \qquad (18)$$

bility of a "hit" is dependent on the number of "hits" recorded already We then have the following

$$\frac{dl_t^x}{dt} = -(\beta + px)\, l_t^x + (\beta + p\ \alpha - 1)\, l_t^{x-1} \qquad (19)$$

This can be integrated to give

$$l_t^x = \frac{l_0\, e^{-(\beta+p\alpha)t}}{\alpha!} \binom{\beta}{p} \left(1 + \frac{\beta}{p}\right) \quad \left(\alpha - 1 + \frac{\beta}{p}\right) (e^{pt} - 1)^x \qquad (20)$$

with

$$\frac{d(\log \mu_t l_t)}{dt} = -(\beta + \alpha - 1\ p) + \frac{(\alpha - 1)\, p\, e^{pt}}{e^{pt} - 1} \qquad (21)$$

Here again the form of equation (21) does not accord with observations from human data

21 In the attempts to fit these forward type formulae to human data it was found (Beard, 1950, 1952c) that satisfactory numerical results could be obtained by expressing $\mu_t l_t$ in gamma function form subject to a terminal age ω, i e the infinite tail of the curve is the opposite way round to what would be considered natural This formula, after elimination of a constant element representing accidental mortality, can be derived from the difference differential equation

$$\frac{dl_t^x}{dt} = pl_t^x - pl_t^{x-1} \qquad (22)$$

27 Calculation of the moments of the distribution of deaths by age for a population of mice (Greenwood, 1928) shows that a Pearson type III (gamma function) would give a fair representation, but, as with the human data, the curve is the "opposite way round", i e subject to a terminal age By inference the Perks (logistic) curve would give a fair representation of this data No calculations have been made on animal data or on physical objects such as electric light bulbs and motor cars (e g Cramer, 1958) but it would seem worth while trying to find out if observed data of this latter type would distinguish between the two types of processes

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may be set up. This has the solution

$$l_t^x = k\,e^{pt}/(1+De^{pt})^{\beta+\alpha+1} \tag{29}$$

If death occurs when the units *fall* below α, we have

$$l_t = \sum_{\alpha} l_t^x = k/(1+De^{pt})^{\beta+\alpha}$$

$$= l_0\, D(1+D)^{\beta+\alpha}/(1+De^{pt})^{\beta+\alpha} \tag{30}$$

We also have

$$\frac{d(\log \mu_t l_t)}{dt} = p - \frac{(\beta+\alpha+1)\; pDe^{pt}}{1+De^{pt}} \tag{31}$$

and

$$\mu_t = \frac{p(\beta+\alpha)De^{pt}}{1+De^{pt}} \tag{32}$$

We have now found *a difference* equation model which *leads* to a Perks (logistic) *formula for* μ_t. In formula (31) the upper limit of μ_t is $p(\beta+\alpha)$, $p \sim 0{\cdot}1$ and the limit $\sim 0{\cdot}7$ so that $(\beta+\alpha) \sim 7$.

25. The distribution of x in the population at age 0 implied by equation (29) is a *decreasing* geometrical progression, i.e.

$$\frac{D}{1+D},\ \frac{D}{(1+D)^2},\ \ldots\ \frac{D}{(1+D)^x}$$

For human mortality D is small (of the order of 10^{-4}) so that the distribution is very slowly decreasing with increasing x.

26. The significant result which emerges from the experiments made *along* these lines is that to provide results which have some reasonable semblance to observed human mortality the backward type of model has to be adopted. This is consistent with death being regarded as the culmination of a degenerative process such that death occurs when the organism reaches a certain level of degeneration. The mathematical models are based *on* numerical results for adult ages and interpolation back to *birth* is possibly a questionable process, a more suitable approach being to regard the life and death process as a period during which the organism is building up to a complex situation with a subsequent degeneration. This would lead to models in which the whole of life process would be looked upon as the resultant effect of two opposing forces.

APPENDIX

27 Calculation of the moments of the distribution of deaths by age for a population of mice (Greenwood, 1928) shows that a Pearson type III (gamma function) would give a fair representation, but, as with the human data, the curve is the "opposite way round", i e subject to a terminal age By inference the Perks (logistic) curve would give a fair representation of this data No calculations have been made on animal data or on physical objects such as electric light bulbs and motor cars (e g Cramer, 1958) but it would seem worth while trying to find out if observed data of this latter type would distinguish between the two types of processes

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